Selenium Supplementation and Cardiovascular Outcome Markers in Hemodialysis Patients: A Randomized, Controlled Trial

by

Elizabeth Jessica Sussman

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Approved April 2013 by the
Graduate Supervisory Committee:

Carol Johnston, Chair
Kenneth Boren
Sandra Mayol-Kreiser
Karen Sweazea
Linda Vaughan

ARIZONA STATE UNIVERSITY

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ABSTRACT

Background Hemodialysis (HD) patients elicit an oxidant-antioxidant imbalance in addition to a selenium deficiency, possibly contributing to cardiovascular disease (CVD) mortality.

Objective To evaluate the effect of selenium supplementation on CVD outcomes and antioxidant status in HD patients.

Design A randomized controlled intervention trial conducted from October 2012 to January 2013.

Participants/setting The study included 27 maintenance HD patients (61.1±17.5y, 14M, 13F) receiving HD in the greater Phoenix, AZ area.

Intervention Patients received one of three treatments daily: 2 Brazil nuts, (5g, 181µg/day of selenium as selenomethionine [predicted]), 1 tablet of selenium (200µg/day of selenium as selenomethionine), or control (3 gummy bears).

Main outcome measures Antioxidant status outcome measures included total antioxidant capacity, vitamin C, and RBC and plasma glutathione peroxidase (GSH-Px). CVD outcomes measures included brain natriuretic peptide; plasma cholesterol, high density lipoprotein, low density lipoprotein, triglycerides; blood pressure, and thoracic cavity fluid accumulation.

Statistical analyses performed Repeated measures ANOVA analyzed changes over time and between groups at months 0 and 2 and months 0 and 3.

Results Independent analysis showed the Brazil nuts provided 11µg of selenium/day and the pill provided 266µg of selenium/day. Consequently, the Brazil nut group was combined with the placebo group. 21 patients completed 2 months of the study and 17

patients completed the study in its entirety. Data was analyzed for months 0, 1 and 2. No significant differences were noted for antioxidant status outcome measures with the exception of plasma GSH-Px. Patients receiving the selenium pill had a significant increase in plasma GSH-Px compared to the placebo group (6.0±11 and -4.0±7.6, respectively, p=0.023 for change between month 0 and month 2). No significant differences were seen in total antioxidant capacity or for CVD outcome measures over time or between groups.

Conclusions These data indicate that selenium supplementation increased plasma GSH-Px concentration in HD patients; however, oxidative stress was not altered by selenium supplementation. The low vitamin C status of HD patients warrants further research, specifically in conjunction with selenium supplementation.

DEDICATION

This dissertation is dedicated to my grandma who always believed that one day, I would write a book. This "book" is for you, grandma. I love you.

This dissertation is also dedicated, in loving memory, to Irving Ruderman. I know my grandpa is looking down, smiling, and thinking "that's my Precious!". I miss you dearly.

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INTRODUCTION

Overview

The escalating number of deaths per year related to kidney disease is troubling. All-cause mortality among current end stage renal disease (ESRD) dialysis patients (adjusted for age and gender) per 1,000 patient years is 154 and 313 for patients aged 45-64y and 65+, respectively.[1] The major cause of death in patients with kidney failure is cardiovascular disease (CVD). In the existing dialysis population, 39% of deaths are attributable to cardiac disease, and in hemodialysis patients, 84.7 deaths per 1000 patient years are due to cardiovascular mortality.[1]

Intervention trials using conventional therapies to improve CVD risk (e.g., lipid lowering medications) have been successful in improving lipid markers, such as plasma low density lipoproteins, cholesterol and triglycerides, however have been unsuccessful in decreasing mortality rates in hemodialysis (HD) patients; however, several trials that utilized antioxidant therapies demonstrated reduced CVD events in HD patients.[2] Antioxidant capacity is impaired in the hemodialysis population[3, 4] leading to elevated oxidative stress.[5] Glutathione peroxidase (GSH-Px) is a potent free radical scavenger that specifically reduces hydrogen peroxide and protects cells from oxidative damage. Plasma GSH-Px is made primarily in the proximal tubular cells of the kidney[6, 7] with small amounts made in other tissue, including the liver, lung, heart, intestine, brain, and skeletal muscle.[7, 8] Once made, it is secreted into the extracellular fluid.[7] Research shows both plasma and red blood cell (RBC) GSH-Px are reduced in patients with chronic kidney disease (CKD) compared to their healthy counterparts.[9, 10] Selenium is required for GSH-

Px function and has also shown to be reduced in HD patients.[11] This deficiency of selenium in HD patients may be due to selenium lost in dialysate[11] however this research is inconclusive.[12]

Selenium is found in foods in two forms: organic and inorganic. The inorganic forms are mainly found in supplements and some plant foods. The three forms of inorganic selenium include selenite, selenate, and selenide. The organic forms are found in food, with selenium replacing the sulfur component of two specific amino acids, resulting in either selenomethionine (plant foods) or selenocysteine (animal foods). Specifically in HD patients, selenium supplementation using an inorganic form of selenium has demonstrated inconsistent results regarding its effect on both plasma and RBC GSH-Px. Several researchers found plasma selenium to increase with selenium supplementation (using the both the organic and inorganic form);[10, 13-15] however, only two studies[10, 16]saw an improvement in red blood cell GSH-Px, while Zachara et al[14] and Temple et al[13] did not find a significant change in plasma GSH-Px after supplementation.

It is believed the results vary because of the selenium form used in supplementation. The above studies have used either inorganic forms of selenium for supplementation, or have used selenium-rich yeast which is rich in selenomethionine. In healthy adults, research has shown selenomethionine supplementation from a natural food source, Brazil nuts, significantly improved whole blood GSH-Px compared to a selenomethionine tablet.[17] A study involving dialysis patients was conducted in Brazil by Stockler-Pinto et al[15] in which selenium supplementation, in the form of selenomethionine from a Brazil nut, showed improved plasma selenium and RBC GSH-Px. While this is valuable information, greater contribution to the literature is warranted, specifically determining if

organic selenium supplementation from food will have significant effects not only on plasma and RBC GSH-Px, but on more specific cardiovascular endpoints, including brain natriuretic peptide (BNP), a hormone used to determine heart failure, along with antioxidant measures, including total antioxidant capacity and plasma vitamin C. Interestingly, previous studies evaluating the effect of selenium supplementation on cardiovascular endpoints in HD patients have not examined its effect on BNP. This knowledge would contribute immensely to the current research, and with the expanded cardiovascular and antioxidant endpoint information, hemodialysis treatment could be significantly altered and decreased mortality is quite possible.

Statement of Purpose

The purpose of this randomized controlled trial in hemodialysis patients is threefold. The first aim is to evaluate the effect of selenium supplementation on cardiovascular disease outcomes in hemodialysis patients. The second aim is to determine the effect of selenium supplementation on antioxidant status in hemodialysis patients. The third aim is to compare the effect of selenium supplementation from a food source versus a supplement on antioxidant and cardiovascular disease outcomes in hemodialysis patients.

Hypotheses

The primary hypothesis of this research is that selenium supplementation compared to control will independently improve cardiovascular disease outcomes in hemodialysis patients as measured by thoracic cavity bioimpedence; brain natriuretic peptide; blood pressure; plasma low density lipoprotein, high density lipoprotein, total cholesterol and triglycerides.

The secondary hypothesis of this research is that selenium supplementation compared to control will independently raise antioxidant status in hemodialysis patients as measured by plasma total antioxidant capacity, plasma vitamin C, plasma glutathione peroxidase, and red blood cell glutathione peroxidase.

The tertiary hypothesis of this research is that selenium from a natural food source (Brazil nut) will independently improve biomarkers, including thoracic cavity bioimpedence; brain natriuretic peptide; blood pressure; plasma low density lipoprotein, high density lipoprotein, total cholesterol, triglycerides; plasma total antioxidant capacity, plasma vitamin C; and plasma and red blood cell glutathione peroxidase in hemodialysis patients to a greater degree than selenium from a supplemental source (selenomethionine tablet).

Definition of Terms

Brain Natriuretic Peptide (BNP): a protein containing 32 amino acids secreted by the cardiomyocytes as a result of increased left ventricular mass; BNP is used to diagnose heart failure.[18]

Glutathione Peroxidase (GSH-Px): a human enzyme responsible for dissipating hydrogen peroxide; GSH-Px is considered the second line of defense in the antioxidant defense system and requires selenium to function.[19]

Total Antioxidant Capacity (TAC): the cumulative action of glutathione, ascorbic acid, vitamin E, bilirubin, trolox, bovine serum albumin, and uric acid in body fluids and plasma.[20]

Delimitations and Limitations

The study was conducted with maintenance hemodialysis patient from the Phoenix, Arizona area. Therefore, the results of the study can only be generalized to a similar patient population. In addition, the study contains a small sample size of 30 participants due to financial constraints. Furthermore, other markers of cardiovascular disease (e.g. Troponin T), measures of lipid peroxidation (e.g. malonlydialdehyde and thiobarbituric acid reactive substances), and vitamin E would have been useful however the cost exceeded financial capabilities.

Chapter 2

REVIEW OF LITERATURE

Free Radicals and Antioxidants

Development of Free Radicals

Oxidative stress can be defined as an excessive production of reactive oxygen species, an extremely reactive molecule, or inadequate removal these reactive oxygen species.[21] Oxidative stress is generally thought of as a negative reaction in the human body. On the contrary, it is necessary for defense against invading microorganisms as well as tissue repair and inflammation. However, in an uncontrolled environment, oxidative stress can be harmful.[22] Briefly, neutrophils and monocytes-macrophages increase oxygen (O_2) consumption, resulting in the formation of superoxide (O_2-). Production of this radical can either be from normal physiologic processes or external stimuli, such as ozone or ethanol.[23] Superoxide can combine with nitric oxide, a reactive nitrogen species,[24] to form other toxic nitrogen species, such as peroxynitrite (ONOO-), or it can be converted to hydrogen peroxide (H_2O_2). Hydrogen peroxide, which is not extremely harmful itself,[19] can then react with intracellular iron to produce hydroxyl radicals (OH-), or it can react with chloride to produce hypochlorous acid (OCl).[22] These reactive molecules (OH-, ONOO-, OCl-, etc), collectively termed reactive oxygen species (ROS), in conjunction with pro-inflammatory cytokines will increase the generation of oxidants, resulting in lipid cell membrane breakdown, DNA damage and protein accumulation.[19]

Antioxidant Defense System

The human body has a strong antioxidant defense system, which helps to dissipate the harmful reactive oxygen species (ROS). The three main enzymatic antioxidant systems are superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px). As a first line of enzymatic defense, SOD, present in the mitochondrial matrix and cytosol, catalyzes the conversion of superoxide (O_2^-) to hydrogen peroxide (H_2O_2) and oxygen (O_2), dissipating the superoxide molecule, a harmful free radical. Catalase and GSH-Px are considered the second line of defense. Catalase works to convert hydrogen peroxide into water (H_2O) and oxygen (O_2) and is found throughout most of the body. GSH-Px is required to reduce organic lipid hydroperoxides as well as hydrogen peroxide.[19] Specifically, GSH-Px, in conjunction with reduced glutathione (GSH), catalyzes hydrogen peroxide to oxidized glutathione and two molecules of water. This reaction can continue only if other vitamins and components are present. Specifically, riboflavin and niacin are necessary for glutathione reductase to reduce oxidized glutathione back to GSH (See figure 1). The human body also has nonenzymatic antioxidant systems to defend against harmful free radicals. The four major nonenzymatic radical scavengers are glutathione, vitamin C, vitamin E, and certain proteins.[19] Glutathione, mentioned above as reduced glutathione, is found in all cell types. It converts hydrogen peroxide to water with the enzyme glutathione peroxidase. Vitamin C is found all throughout the body[19] and is a potent antioxidant. Vitamin C, or ascorbic acid, reduces free radical molecules, resulting in semidehydroascorbic acid,[19] followed by formation of dehydroascorbic acid. GSH, in conjunction with the enzyme dehydroascorbate reductase, regenerate ascorbic acid by reducing dehydroascorbic acid. The regeneration of ascorbic acid allows the

molecule to continue to neutralize free radicals.[25] Research has shown vitamin C supplementation maintains blood glutathione levels and improves antioxidant capabilities in the blood.[26] Vitamin E is found in cell membranes and protects cells against lipid membrane peroxidation. Specifically, it protects the unsaturated fatty acids in the phospholipids of the cell membrane.[25] Using the reducing potential of a hydroxyl group (-OH), vitamin E can inhibit the damaging radical cascade that takes place in the lipid membrane, most notably the peroxyl radicals.[25] The result is a vitamin E radical that must be reduced in order to be reused. The recycling of vitamin E is dependent on reduced glutathione and vitamin C.[25] Figure 2 demonstrates the synergism of glutathione, vitamin C and vitamin E as antioxidants. Lastly, certain proteins, including albumin, transferrin, ceruloplasmin and ferritin, can aid in the antioxidant process.[19] Albumin is found in the plasma and plays a large role in binding and transporting molecules throughout the body. Albumin binds a host of molecules, including the divalent cations calcium and magnesium, bile acids, zinc, copper, and folate, to name a few.[27] Transferrin is a transport protein that transports iron in the oxidized, ferric (+3) form. Ceruloplasmin is a copper-containing enzyme responsible for converting iron into the ferric from in order to bind to transferrin, whereas ferritin stores iron in the ferric form until the body requires its use. These proteins aid in limiting oxidative stress by binding transition metal ions, such as ferric iron, to minimize the production of free radicals.[19] The intricate antioxidant system the human body has developed is complex and interdependent on many nutrients.

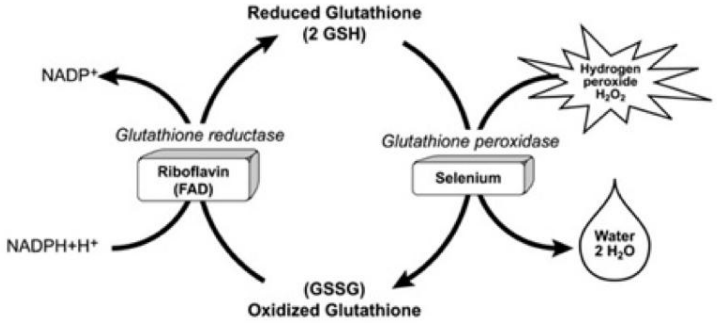


Figure 1. The Glutathione Oxidation Reduction Cycle[a]

[a]Image courtesy of Linus Pauling Institute, Oregon State University, Corvallis, OR

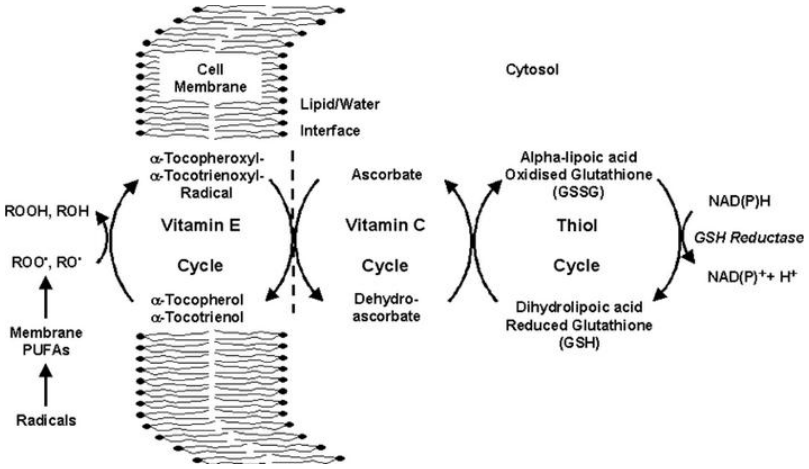


Figure 2. The interaction between glutathione, vitamin E and vitamin C in the antioxidant defense system[b]

[b]Image courtesy of Me van Meeteren et al[28]

Selenium

Selenium Nutriture

Selenium is a nonmetal that is required for a number of enzymatic, namely antioxidant, functions in the human body[25] making it an important nutrient for controlling oxidative stress levels. Selenium can be found in both plant and animal sources. There are two forms of selenium: organic and inorganic. The two organic forms are selenocysteine and selenomethionine. Of the twenty amino acids, methionine and cysteine are the only sulfur containing amino acids. The sulfur incorporated in each of these amino acids can be displaced by selenium, resulting in either selenocysteine or selenomethionine (See figure 3). Selenocysteine and selenomethionine are typically found in animal and plant products, respectively.[25] Because plants do not require selenium, the element is incorporated into methionine by substituting selenium for sulfur, resulting in selenomethionine.[29] The amount of selenium from a plant or plant food product varies significantly depending on the soil. Selenium is found in the soil and transferred to the plant product when it grows.[29] Therefore, depending on where the plant grows, the amount of selenium in the product can differ drastically[30] – up to a ten-fold difference.[29] Consequently, the animal eating the plant will also ingest a varying amount of selenium due to selenium soil content.[29, 30] The amount of selenium from animal products, typically as selenocysteine, also differs significantly due to the varying amount of selenium ingested when the animal ate the plant product.[29] As mentioned above, animal products typically contain selenocysteine as the main source of selenium. It is important to note this is the biologically active form in animals and humans.[29] The inorganic forms of selenium include selenite, selenate, and selenide. These forms of selenium can be found

in certain vegetables, however they are mostly found in supplements, typically in the form of selenate, selenite or selenomethionine.[25] The recommended dietary allowance for selenium is 55µg/day for adults.[31]

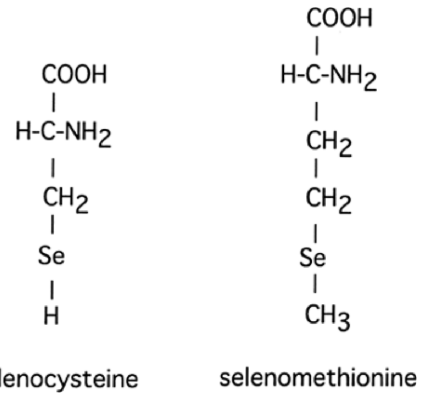


Figure 3. Sulfur containing selenocysteine and selenomethionine[c]

[c] Image courtesy of Shils et al [32]

The bioavailability of selenium differs with source; however, overall the mineral is highly available. Approximately 80 percent of organic selenium is absorbed, primarily in the duodenum with small amounts absorbed in the jejunum. It is thought that selenomethionine is better absorbed than selenocysteine[25] and that the absorption follows the same pathway as methionine. Among inorganic forms of selenium, selenate is better absorbed than selenite however selenite is better retained in the body compared to selenate.[33]

After initial ingestion, the various forms of selenium are metabolized differently. Specifically, selenium ingested as selenomethionine has three fates: it can be used for protein synthesis, recognized as methionine and stored in the amino acid pool, or catabolized to selenocysteine.[25] The other organic form of selenium, selenocysteine (from food consumption or degradation of selenomethionine), may be catabolized to free

selenium which is then reduced to form selenide.[25] Selenide has one of two fates: it will be phosphorylated to selenophosphate, or methylated to be excreted in the urine. If converted to selenophosphate, the molecule will be used for the synthesis of selenocysteine in the liver.[34] In fact, selenophosphate must undergo a reaction with serine to form selenocysteine which can be added to peptide chains of specific selenium-dependent enzymes, including GSH-Px.[25] Ingested selenocysteine cannot be used directly for selenium-dependent enzyme synthesis, also called selenoproteins, however it can be incorporated into selenium-containing proteins.[35] A selenoprotein is one in which endogenously synthesized selenocysteine is formed and incorporated into proteins, whereas a selenium-containing protein is one in which selenium is not in its biologically active and functional form (i.e: selenomethionine).[36] The inorganic forms of selenium must be converted to selenophosphate to be used for selenoprotein synthesis in the liver.[34]

Selenium is mainly found in the kidney and liver with smaller amounts in the heart muscle, skeletal muscle, brain and nervous tissue. When selenium intake is low, the liver and blood amounts tend to decrease first whereas kidney concentrations are not as affected.[37] Selenium is stored in two pools in the human body.[29] The first is selenium as selenomethionine, which is dependent on dietary intake and is not dependent on the body's need for selenium, but rather a function of methionine turnover.[38] The second body pool is located in the liver[29] and is termed the exchangeable metabolic pool, in which all forms of selenium that contribute to functional uses of selenium containing compounds are stored, including GSH-Px and selenoprotein P.[34] Interestingly, as selenium intake decreases, synthesis of liver GSH-Px is reduced, allowing selenium availability for synthesis of other selenoproteins.[29] Approximately 10% of selenium is

excreted in feces with the majority of the remainder excreted in the urine as urinary metabolites.[39]

Selenium in the Antioxidant Defense System

Selenium is a pivotal part of the human antioxidant system. As mentioned above, GSH, or reduced glutathione, is needed to reduce hydrogen peroxide resulting in water and oxidized glutathione. This reaction cannot occur without the enzyme GSH-Px which is dependent on selenium.[40, 41]. GSH-Px is found throughout the body, including the red blood cells, lungs, heart, kidney and liver.[42] Research has shown a deficiency in either selenium or GSH-Px will result in membrane damage and protein dysfunction.[43] As previously mentioned, vitamin C and E play a crucial role in antioxidant function in association with GSH. Furthermore, the regeneration of both vitamin E and C require GSH and glutathione dehydrogenase. Hence, adequate vitamin C status will spare GSH and help to regenerate vitamin E thereby maximizing antioxidant protection[44]; a deficiency in vitamin E can result in neurological damage[45] while a deficiency in vitamin C can be fatal.[46]

In addition to GSH-Px, selenium is required for a number of other molecules in the body. Of the three types of deiodinases, which are responsible for thyroid hormone activation and deactivation, selenocysteine has been shown to be the active site of type 1 deiodinase and type 3 deiodinase. These deiodinases are responsible for regulating activation and deactivation of 3,4,3'-triiodothyronine, or T3, which is involved in brain development and growth.[47] Selenocysteine is also the active site for selenoprotein P, which is comprised of ten selenocysteine residues[48] and is the major selenium containing protein in the plasma.[25] While once thought of as a selenium transport protein,

selenoprotein P is a glycoprotein that functions as an extracellular antioxidant dissipating peroxynitrite, a reactive nitrogen species.[49] Finally, thioredoxin reductase is an enzyme containing selenium in the form of selenocysteine, required for the growth of cells.[48]

Selenium Toxicity

While diminished levels of selenium can be detrimental to the body, toxic intake of selenium can also be deleterious. The Dietary Reference Intake has set the upper limit for adults for selenium consumption at 400µg/day due to brittleness and loss of hair and nails.[31] At very high levels, selenium can be considered a prooxidant inducing oxidation.[50] Certain selenium compounds can induce generation of superoxide while others do not. The selenium compounds that result in the generation of superoxide include selenite, selenocysteine, and selenium dioxide, while selenate, selenomethionine, and elemental selenium do not induce the generation of superoxide.[50-52] The inorganic forms of selenium react with tissue thiols[53] resulting in selenotrisulphides. The newly formed selenotrisulphides further react with the oxidized thiols forming superoxide.[50] Another plausible reason for selenium causing oxidative stress suggests higher levels of selenocysteine inhibit the methylation of selenium for urine excretion, causing an increased concentration of the hydrogen-selenide, resulting in the formation of superoxide.[50] Lastly, selenium may assist in reactions leading to the formation of reactive selenium-containing intermediates.[54] Nonetheless, selenium toxicity is a result of increased oxidative damage exceeding the capacity of the antioxidant defense system[52] causing DNA damage.[55]

While selenium is valuable to sustain the antioxidant defense system, it can also be a prooxidant, increasing oxidative stress. While this sounds detrimental, it may be a

benefit to the body. In fact, researchers have suggested selenium has the potential to be used in conjunction with anticancer drugs or radiation to improve the efficacy of the treatment, in addition cancer prevention.[56] The organic form of selenium, specifically selenomethionine, when injected into human tumor cell lines, including breast carcinoma, prostate cancer cells and melanoma, was found to causes apoptosis, or cell death.[57] Further, when evaluated in vitro on human breast carcinoma cells, the tumorigenic mammary epithelial cells were highly sensitive to selenocysteine and selenomethionine, resulting in apoptosis. Interestingly, the non-tumorigenic cells did not experience apoptosis until a significantly higher dose was given.[58] Additionally, selenium has been shown to suppress melanoma cells while inhibiting the growth of tumors in the lung, also due to apoptosis.[59] Conversely, research has also shown selenium to be detrimental as a prooxidant. In a 12 day trial, mice were supplemented with selenite. Results showed selenium supplementation increased malonyldialdahyde, a measure of lipid peroxidation, and decreased glutathione, and the antioxidants superoxide dismutase and catalase.[60] For otherwise healthy adults, when evaluating serum selenium concentration and mortality, a recent review suggest a U-shaped link such that those with a high selenium status and low selenium status have increased all-cause mortality. Additionally, supplementation of those with a relatively high level of serum selenium may increase their risk of type 2 diabetes.[61]

Selenium and Cardiovascular Disease

Selenocysteine, the biologically active form of selenium and sometimes referred to as the 21st amino acid,[62] is required for many enzymatic, namely antioxidant, functions in

the human body as discussed previously. A deficiency in the above trace element will produce harmful effects, including cardiovascular disease, in otherwise healthy adults.

Two diseases have been linked to and caused by a selenium deficiency: Keshan Disease (KD) and Kashin-Beck Disease (KBD). KD results in cardiomyopathy whereas KBD results in osteoarthropathy. KD dates back to 1935 when an outbreak occurred in Keshan County, China. The effects of the disease resembled the plague and mostly affected women of child-bearing age and infants after weaning.[63] During the 1960's, a selenium deficiency occurring in livestock, called white muscle disease (WMD), was also observed in the areas of those affected with KD. Researchers noticed the similarities between WMD and KD, supplemented human patients with selenium, in the form of selenite, and vitamin E in the hopes that KD would be prevented; however, no clear results were extracted from study.[63] More research was conducted in the early 1970's. Researchers measured blood and hair selenium concentrations and collected diet records to determine selenium intake and saw a connection between KD and selenium deficiency.[63] Further research showed hair selenium,[64] and selenium concentration of the heart, muscle, liver and kidney[65] of the affected area was significantly lower than non-KD affected areas. To further research this problem, scientists gave high-risk children either sodium selenite tablets or placebo. The children receiving the treatment had significantly less death than those receiving the placebo.[64] It is worth mentioning the severity of KD was shown to be proportional to the extent of the selenium deficiency.[66]

Researchers have concluded that while selenium is essential, it is not the only cause of KD. This is evident by hair selenium of various parts of China in which areas that developed KD had the same low selenium status as areas that did not develop KD.[66]

Additionally, a seasonal variation was seen with KD development.[66] It is now accepted that a selenium deficiency plays a major role in KD as well as coxsackie virus infection. Of the sixteen coxsackie viruses, coxsackie virus B3 has been shown to contribute to the development of KD, resulting in myocarditis.[67]

Currently, selenium deficiency is rare among most of the world, and only seen in KD affected China.[63] As mentioned above, selenium is crucial for antioxidant function and as expected, the person infected with KD experiences an extreme impairment in their antioxidant defense system due to the selenium deficiency. They also experience death of the myocardium as an outcome of injury to cell membranes and proteins.[63] Although KD is now considered rare, it is still noteworthy to evaluate if selenium status can predict and/or prevent heart disease.

Many different types of studies have been conducted to evaluate if selenium status is related to heart disease and death in participants without KD, however the results vary widely and are inconclusive. In a cross sectional study, Kok et al compared plasma, red blood cell and toenail selenium in participants with and without acute myocardial infarctions from the Netherlands. They found levels of all three markers to be significantly lower in those with acute myocardial infarctions than those without. Additionally, the authors noted the diminished selenium levels were present before the infarction occurred as evidenced by the low toenail selenium levels, as toenail selenium is reflective of long term status, up to one year. These data indicated that selenium contributes to CVD etiology.[68] A longitudinal study involving over 8000 men and women of Finland investigated serum selenium and risk of death. The study used data from patients who died from any type of heart disease or experienced a non-fatal myocardial

infarction over a seven year follow up period, and compared the values to matched healthy controls. The results showed those who died of heart disease or had a non-fatal MI had significantly lower serum selenium. Additionally, the relative risk associated with a serum selenium of <45µg/l for CHD death, CVD death, and fatal and non-fatal MI were all 2 times more likely than that of healthy controls ($p<0.01$). Additionally, serum selenium was attributed to 22% of deaths in the study population.[69] In a prospective study, Wei et al found no significant increase in death due to heart disease or stroke in relation to serum selenium at baseline after a 15 year follow up period in over 1100 subjects.[70] In a thirteen-year intervention period, researchers supplemented patients free from cardiovascular disease with 200µg selenium/day as selenium-rich yeast. Cardiovascular disease and mortality was assessed however no statistical significance was found between treatment and above mentioned CVD endpoints. It is noteworthy that this study was a subset of the Nutritional Prevention of Cancer Trial and thus, while participants were free from CVD, they had a history of nonmelanoma skin cancer within one year of randomization.[71] Finally, Flores-Mateo et al conducted a meta-analysis evaluating the association between selenium biomarkers and coronary heart disease endpoints using observational studies, and the effectiveness of selenium supplementation in coronary heart disease prevention using randomized trials. Observational study results showed a 50% increase in selenium concentrations was associated with a 24% reduction in risk of coronary heart disease. Results of the randomized controlled trials showed an 11% decrease risk of coronary heart disease when comparing selenium supplements to placebo, although not significant. The authors suggest that while the observational studies showed an inverse relationship between selenium and coronary heart disease, and only a

few randomized control trials have been conducted regarding selenium and coronary heart disease with inconsistent results, more research is needed and selenium supplementation should not be recommended at this time.[72]

Selenium is an integral part of the human antioxidant system and a deficiency in the mineral causes severe problems as evidenced by the cardiomyopathy seen with KD affected people. However, selenium supplementation trials have not shown an overwhelming response and subsequent decrease in heart disease. It may be that supplementation is only effective in those with a deficiency.

End Stage Renal Disease

End Stage Renal Disease, Cardiovascular Disease and Antioxidant Status

Dialysis patients present with many physiological problems due to the nature of their disease and the diminished homeostatic regulatory ability of the kidney. Besides a kidney transplant, two dialysis treatment options are available: hemodialysis and peritoneal dialysis. Hemodialysis (HD) uses an extracorporeal filtration system to remove toxic waste from the body, and peritoneal dialysis uses the individual's peritoneal cavity as a filter to remove toxic waste. While treatment, specifically HD, can be an effective tool in removing harmful waste products, it can also be detrimental leading to cardiovascular disease (CVD).

End stage renal disease (ESRD) patients present with uremia, or buildup of nitrogenous waste products, including urea nitrogen and creatinine, in the blood. To remove these harmful toxins, dialysis or transplantation is needed. Hemodialysis is the most common form of treatment to date.[1] The patient's blood is circulated through an extracorporeal filtration system and returned to the body during the HD treatment. While

this process removes urea from the body, it does not correct all abnormalities seen with ESRD and it has been suggested to cause increased oxidative stress and decreased antioxidant capacity, leading to other harmful diseases, such as CVD. CVD development starts with oxidation of lipoproteins in the arterial wall and the release of malondialdehyde (MDA), short chain aldehydes that are byproducts of lipid peroxidation, along with other aldehydes. These molecules can alter residues of apolipoprotein B, a component of low density lipoproteins (LDL). As a result, the newly altered LDL contributes to the formation of foam cells by being consumed by macrophages in the subendothelial space.[73, 74] This, in turn, triggers a series of reactions to initiate atherosclerotic plaque formation. Over time, the narrowing of the arteries diminishes blood flow to vital organs, including the heart and kidney.[73]

Research has shown both antioxidant pathways (enzymatic and non-enzymatic) in patients with chronic renal failure and on dialysis to be significantly impaired.[3] Specifically, serum selenium, glutathione peroxidase, and vitamin C were lower in chronic renal failure (CRF) and HD patients compared to healthy controls. Interestingly, this decrease was exacerbated by the treatment of HD as patients with HD treatment had lower serum levels of glutathione peroxidase and vitamin C, and higher levels of MDA compared to CRF patients.[3] Moreover, the dialysis process induces oxidative degradation of membrane lipids.[75] With respect to vitamin C, Morena et al found ~65mg of vitamin C was lost each dialysis session,[76] exacerbating the risk for low antioxidant status. Additionally, Koenig et al[77] found the same to be true: HD patients presented with an impaired free radical scavenger system with increased MDA. Furthermore, they also

found the enzymatic antioxidant pathway to be significantly impaired as evidenced by decreased RBC GSH-Px.

Impairment of the Oxidant-Antioxidant Balance

It has been suggested the greater impairment of the oxidant-antioxidant balance in HD patients compared to CRF patients is because the process of an extracorporeal filtration system (hemodialysis) results in blood membrane interactions and volatile hemodynamic conditions, both of which can be harmful to the homoeostasis of the dialysis patient.[78] The filtration system of the hemodialysis process removes uremic toxins, but glucose, vitamin C, amino acids, and small peptides are also removed.[78] The removal of the latter substances is undesirable and is a contributor to oxidative damage and subsequently, CVD. For HD patients, there are different external membranes to filter the blood. Yavuz et al found the polysulfone membrane to cause more oxidative stress compared to using a hemophan membrane as evidenced by an increased serum MDA concentration, and a greater decrease in GSH-Px and selenium after dialysis treatment in the patients using the polysulfone membrane.[75] Interestingly, these results are inconsistent with previous research. Cristol et al found that after only 15 minutes of HD treatment, leukocytes and monocytes were activated with a cuprophane filter but not with a polysulfone filter.[79] Another plausible reason for the higher incidence of oxidative stress in HD patients is the presence of lipopolysaccharide (LPS), a harmful endotoxin. Research suggests LPS in the dialysate could contribute to free radical production by activation of monocytes/macrophages.[80] Lastly, oxidative stress in the dialysis patient may be, in part, attributable to adjuvant pharmacotherapy. Iron and erythropoietin (EPO) are typically given intravenously during each dialysis treatment session. Unfortunately,

while needed, the administration of both iron and EPO augment oxidative stress independently.[81, 82]

This increase in oxidative stress with decreased immunity among HD patients is a basis for cardiovascular disease development. Cardiovascular disease, development described above, remains the primary cause of death for HD patients. The 2011 US Renal Data System Annual Report[1] shows from 1997 to 1999, the percentage of deaths due to cardiac disease was 45. The 2007 to 2009 data shows this percentage has decreased to 39, although cardiac death still remains the primary cause of death. Efforts have proved useful as there was a 29% decline over the last ten years in CVD mortality among HD patients. Even so, the oxidant-antioxidant imbalance persists, resulting from a damaged radical scavenger system and increased production of free oxygen radicals,[78] resulting in death.

Selenium and End Stage Renal Disease

Selenium Status of End Stage Renal Disease Patients and its Impact on Health

There is a large amount of research that has evaluated the selenium status in dialysis patients as compared to healthy subjects. In this patient population, selenium has been evaluated in both serum and plasma. Briefly, RBCs are absent from both plasma and serum, and plasma contains clotting factors while serum does not. Additionally, plasma and serum contain proteins (such as albumin), hormones, minerals, electrolytes, carbon dioxide, etc. As shown below, researchers use plasma, serum or RBC selenium to determine selenium status. This may, however, not be the best way to determine selenium status. Biologically active selenium is found as selenocysteine as part of enzymes requiring the compound, such as glutathione peroxidase, and measuring these enzymes is a functional and more highly sensitive method of selenium status.[83]

An overwhelming majority of the research demonstrates both HD and PD patients present with decreased serum or plasma selenium compared to matched healthy subjects. Specifically, Bonomini et al, Zachara et al, and Foote et al have used cross sectional data to compare plasma selenium in HD patients to those of healthy controls; they have all found those of hemodialysis patients to be significantly lower.[14, 84, 85] Additionally, Antos et al, Bogye et al, del Moral et al, and Pakfatret et al found similar results when comparing serum selenium of HD patients to those of healthy subjects in cross sectional data.[11, 86-88] These results are not different when evaluating the difference between healthy adults and those undergoing PD. Apostolidis et al and Pakfetrat et al found patients on PD had markedly lower serum selenium than healthy controls.[11, 89] Interestingly, one study[89] found a decrease in serum selenium, mentioned above, however

concluded there was no selenium deficiency in this population because the average adjusted serum-transported selenium per liter of blood was not different from the healthy controls. This was the only study to adjust for serum-transported selenium per liter of blood and the only study to date suggesting there is no discrepancy between PD patients and healthy people in regards to serum selenium status. Alternatively, Charney et al did not find a significant difference in RBC selenium values in HD patients when compared to healthy controls.[90] When comparing different dialysis modalities (HD and PD) in relation to selenium status, Pakfetrat et al found PD patients had lower serum selenium compared to HD patients, suggesting selenium deficiency is more prevalent in PD patients.[11] Additionally, Dworkin et al found whole blood selenium was lower in PD patients than HD patients.[91]

One plausible reason for this marked decrease in plasma and serum selenium in dialysis patients is the loss of selenium in spent dialysis fluid. The limited amount of research is inconclusive. In hemodialysis patients, Pakfetrat et al found greater amounts of selenium in spent dialysate compared to fresh dialysate, suggesting selenium was lost through the HD dialysis membrane.[11] Additionally, Bogye et al found protein along with selenium in the spent dialysate suggesting selenium is lost due to protein permeability in the HD polysulfone membrane.[87] Along with this loss of protein and selenium in the spent dialysate, the researchers found no change in serum selenium prior to and after dialysis. However, they did find a significant increase in total serum protein after dialysis treatment, regardless of the increase in selenium and protein in spent dialysate. The authors suggest that this increase in serum protein is a result of hemoconcentration, a decrease in plasma volume after dialysis. On the other hand, Zachara et al reiterated 95%

of plasma selenium is found bound to proteins that do not cross the permeable membrane of the dialysis filter and thus, selenium cannot be lost during dialysis treatment.[92] In PD patients, the research is limited yet more convincing. Pakfetrat et al evaluated the spent dialysate selenium concentration in PD patients, finding the amount of selenium in spent dialysate was undetectable.[11] Additionally, when comparing fresh dialysate to spent dialysate in PD patients, Sriram et al found no change in dialysate selenium concentration.[93] It is important to mention that while selenium is not found in spent dialysate, researchers have found those patients receiving PD had a greater selenium deficiency compared to HD patients (shown above). As mentioned above, selenium is mostly found as selenomethionine or selenocysteine in the blood and tissues. Additionally, selenomethione has a high tendency to replace methionine in the amino acid pool. Because many trace elements, including selenium, are bound to protein (e.g. selenomethionine), it has been suggested that PD patients could experience a greater loss of protein and thus, trace elements compared to HD patients.[93] Lastly, another valid question concerning the altered serum and plasma selenium values of dialysis patients revolves around the reason for the altered concentration. Research has yet to demonstrate the biological mechanisms of if/how progressive kidney failure causes altered selenium status. Diskin et al suggest the selenium deficiency is a result of the primary disease, kidney failure, causing biochemical alterations.[94] This biological and mechanistic information would contribute immensely to the body of literature and provide greater insight into the disease.

To date, the majority of researchers agree that the selenium status of both PD and HD patients is inferior to those of their healthy counterparts. The next logical, and

important, concern would be to determine if the delinquency is detrimental to health. Researchers agree this to be case. Specifically, del Moral et al state the lower serum selenium levels of HD patients significantly increases the risk for CVD.[88] Additionally, selenium deficiency in uremic, or end stage renal failure, patients contributes to cardiovascular disease, changes in immune function, and skeletal myopathies.[89, 91, 95, 96] While the exact mechanism is not known, it can be hypothesized that because the regulation of selenium stores is through the kidneys,[91] disruption to this organ will alter selenium homeostasis.

While it is clear selenium status is decreased in both HD and PD patients compared to healthy people and this deficiency is involved with cardiovascular disease, the mechanism by which this occurs is unclear. Additionally, the cause of the selenium deficiency is still vague and warrants further research.

Selenium Supplementation and End Stage Renal Disease

It is well recognized that end stage renal disease patients requiring dialysis are at increased risk for CVD.[97] In fact, studies have shown both PD and HD patients to have decreased antioxidant status compared to their healthy counterparts. Specifically, Koenig et al[77] found HD patients present with significantly elevated MDA, superoxide dismutase (SOD) and catalase. The authors proposed the constant increase in SOD and catalase is a result of the continual battle with active reactive oxygen species. Additionally, Capusa et al[98] showed HD patients had significantly increased plasma thiobarbituric acid-reactive substances (TBARS, a consequence of lipid peroxidation), and both HD and PD patients had decreased total antioxidant activity and residual antioxidant activity compared to healthy individuals. For HD patients, it has been suggested that the production of toxins

as a result of the HD filtration membrane is a contributor to the decrease in patients' antioxidant status,[19] in addition to the chronic uremic state.[77] Part of the enzymatic antioxidant defense system in the body includes the enzymatic action of GSH-Px which catalyzes the reaction of hydrogen peroxide to water, and requires selenium for its action. In addition to the low antioxidant status, many cross-sectional studies have revealed dialysis patients present with decreased plasma, serum and RBC selenium,[11, 14] and decreased plasma and red blood cell GSH-Px compared to healthy counterparts.[10, 77]

Unfortunately, relative to the amount of cross-sectional studies, very few selenium intervention studies have been performed. In fact, to date, only eight intervention studies have been completed, and all used HD patient participants. When evaluating plasma selenium change after selenium supplementation, it is not surprising all studies found an increase in plasma selenium after supplementation, as selenium is highly absorbed in the gastrointestinal tract. Of the eight intervention studies, five supplemented HD patients with selenium as either selenite or selenate,[10, 16, 77, 84, 99] a form commonly found in selenium supplements. These studies ranged from 2 weeks[99] to twenty-four weeks.[16, 84] Three of the five studies gave selenium supplementation during dialysis treatment (3 times per week); however, Temple et al[99] provided IV liquid nutrition (which included the supplemented selenium) as the only form of nutrition for the 2 week trial period. In those studies that measured GSH-Px (both plasma and/or RBC), the results varied. For example, Bonomini et al[84] found an increase in whole blood GSH-Px with selenium supplementation compared to control, while Richard et al and Saint-Georges et al found an increase in both plasma and RBC GSH-Px.[10, 16] Koenig et al[77] found RBC GSH-Px and RBC selenium significantly increased after eight weeks of selenium supplementation.

However, Temple et al[99] did not find any change in plasma or RBC GSH-Px after selenium supplementation. One plausible reason for these varying differences in GSH-Px is the location of the enzyme. Extracellular GSH-Px is found in the plasma while cellular GSH-Px is found in the RBC. Many of the studies measured RBC GSH-Px, which tended to increase after supplementation. Plasma, or extracellular, GSH-Px is synthesized mostly in the kidneys.[6] It has been suggested that the reason the researchers are not seeing the desired increase in plasma GSH-Px is because the damage to the kidneys will not allow synthesis of the enzyme regardless of selenium status.[12, 14]

Two of the seven intervention studies used selenium-rich yeast for supplementation, which contains selenium as selenomethionine, an organic form of the element. Zachara et al[14] provided patients selenium-rich yeast tablets for 12 weeks and found supplementation increased plasma selenium but did not significantly change plasma GSH-Px. In another study by the same authors,[12] patients were given selenium-rich yeast tablets or the selenium-rich yeast tablets plus erythropoietin (EPO, a hormone stimulating red blood cell development) for 12 weeks. Results showed selenium treatments (with or without EPO) increased whole blood, plasma, and RBC selenium. An increase in RBC GSH-Px was seen initially but fell to baseline values by the end of the study, whereas plasma GSH-Px did not increase significantly in either group. All of the studies above supplemented with either selenite, an inorganic form of selenium, or selenium rich yeast (assumed to be selenium as selenomethionine). An overwhelming percentage of food selenium is in the form of selenomethionine or selenocysteine while supplemented selenium is typically an inorganic selenium source. This may be the key factor in determining effectiveness of selenium supplementation in dialysis patients.

To date, one study has used a selenium rich food source as its selenium source for supplementation. Stockler-Pinto et al[15] provided hemodialysis patients with one Brazil nut per day for 12 weeks. Results of the study showed an increase in plasma and RBC selenium as well as RBC GSH-Px after selenium supplementation. The authors discontinued supplementation after 12 weeks however followed up with the patients 1 year later and found plasma selenium levels had decreased since supplementation discontinuation however the values did not reach as low as initial study commencement.[15]

Currently, selenium supplementation studies have not been performed with peritoneal dialysis. Additionally, long-term selenium supplementation studies have not been carried out to evaluate effectiveness on oxidative stress, cardiovascular disease outcomes/measures, or toxicity in hemodialysis and peritoneal dialysis patients. More research is needed using naturally high food sources for selenium "supplementation" within the dialysis population. Moreover, future studies need to evaluate long-term effects on endpoints reflecting mortality, specifically cardiovascular mortality. This necessary research will provide insight to sustain or alter current nutrition recommendations for peritoneal and hemodialysis patients. It is worth noting that most multivitamins prescribed for dialysis patients, including Nephrocaps®, Nephrovite®, and Dialyvite® to name a few, contain vitamin C, a water soluble vitamin and potent antioxidant, while only some contain vitamin E, another antioxidant. Interestingly, neither Nephrocaps® nor Nephrovite® contain any selenium while four of the ten formulations of Dialyvite® contain a trace amount of selenium. Current renal nutrition guidelines do not discuss selenium or selenium supplementation[100] and in light of the

information above, the lack of recommendation should be reevaluated upon further testing.

Chapter 3

METHODS

Study Design and Subjects

This study was a randomized, controlled trial using maintenance hemodialysis patients from the greater Phoenix, Arizona area. The study was approved by the Arizona State University Institutional Review Board (see Appendix A for Institutional Review Board approval). In addition, Southwest Clinical Research Institute provided written consent stating the Arizona State University Institutional Review Board process is sufficient for the research to be conducted in Southwest Kidney Institute and Davita dialysis clinics (see Appendix A). The goal was to enrolled thirty subjects for the study. Previous literature enrollment for selenium supplementation in hemodialysis patients ranged from 5 to 81 participants, with an average of 33 participants. Sample size calculation using 80% power, α at 0.05, standard deviation of 40 (based on previous literature) and a difference of means set at 75 (based on previous literature) determined a total of 6 patients per group were required for this parallel design study. Recruitment took place at four dialysis facilities throughout the Phoenix, AZ and surrounding areas. Upon initial analysis of laboratory values from recruitment period, fifty six patients were identified as potential participants. Further inquisition excluded twelve of the fifty six participants due to possession of one or more exclusion criteria (discussed below). Therefore, forty-four participants were spoken to by the study investigator inquiring about involvement in the study. Discussion included study objectives, study procedure, and risks and benefit of the study. Thirty one patients decided to enroll in the study at which time they signed the consent form and were given a copy to keep.

Participants were maintenance hemodialysis patients, defined as greater than 90 days on hemodialysis and hemodynamically stable. Eligible participants must have met the following criteria: a fistula or graft for hemodialysis access, $\geq$18 y of age, potassium value $\leq$ 5.5mg/dL for at least 3 months, albumin $\geq$ 3.3mg/dL for the month of screening, BMI (using estimated dry weight for the month of screening) $\leq$ 40kg/m^2, and free from HIV/AIDS, cancer and Hepatitis C. In addition, patients with antioxidant vitamin supplement usage within the past 90 days and/or smoking history were excluded.

After the consent was signed by all participants, one subject revealed she smoked and thus, was dropped from the study. Therefore, thirty participants were randomized such that ten participants were to consume either two Brazil nuts per day for three months, swallow one tablet per day for three months, or consume three gummy bears per day for three months.

Prior to study commencement, three patients dropped out from the study: two patients decided they did not want to participate and one subject passed away. Between the start of the study and the end of the first month, five participants dropped out; one patient experienced itching, two did not want to participate anymore, one received a kidney transplant, and one left the country for a family emergency. Between the first and second month, one participant dropped out because they did not like the taste of the gummy bears. Lastly, between the second and third month, one patient dropped out because of cancer development, one patient was lost to follow up, one patient no longer wanted to participate, and one patient started the study late (at month 1). Seventeen patients completed the study.

Treatment

Each dialysis patient was randomized to receive one of three treatments for the 3-month intervention: 2 Brazil nuts (5g, estimated to provide 181µ/day of selenium as selenomethionine), 1 tablet of selenium (200µ/day of selenium as selenomethionine), or control (3 gummy bears/day).

Protocol

After the consent was signed by the participant, a health history questionnaire was completed (see Appendix B). The study lasted three months and the four remaining visits were designated M0, M1, M2, and M3 to coincide with the participants regularly scheduled monthly blood draw day at the dialysis center. HD patients follow one of two dialysis treatment schedules: Monday/Wednesday/Friday or Tuesday/Thursday/Saturday. Five of the 30 patients enrolled in the study had their regularly scheduled blood draw day on Wednesday/Thursday while the other 25 patients had their scheduled blood draw day on Monday/Tuesday. The difference in absence of treatment over a 48 or 72 hour period will be analyzed to confirm the lack of effect on outcome variables. At visit M0 (baseline), participants were given a compliance calendar (see Appendix B) and asked to place an "X" on each day they took the food product or supplement. During the four monthly visits, thoracic cavity fluid accumulation was measured by biothoracic impedance prior to dialysis treatment. During the M0, M1 and M2 visits, participants were provided with their experimental product. At each visit (M0, M1, M2 and M3), after the patient had their dialysis lines put into their arm by their respective patient care technician (PCT) or registered nurse (RN), three vials of blood were collected by the RN

or PCT and given to the study investigator for processing. Additionally, at visits M1 and M3, participants received a $15 gift card to Target® for their participation.

Blood Collection and Laboratory Analysis

Blood was collected at four time points throughout the study: month 0, 1, 2, and 3, and was collected immediately before the participants' dialysis session. Three tubes were collected from each patient. The 7mL EDTA tube was stored at room temperature while the 4mL EDTA and 7mL Sodium Heparin tubes were stored in the refrigerator. Once all subjects' blood was collected, it was taken to the ASU laboratory for processing.

Biomarkers measured using commercial ELISA kits included plasma and red blood cell glutathione peroxidase (Cayman Chemical, Ann Arbor, MI, www.caymanchem.com/catalog/703102), brain natriuretic peptide (RayBiotech, Norcorss, GA, www.raybiotech.com/human-bnp-eia-kit.html), and total antioxidant capacity (Cayman Chemical, Ann Arbor, MI, www.caymanchem.com/catalog/709001). Plasma vitamin C was assessed using the 2,4, di-nitrophenylhydrazine spectrophotometer method. Plasma high density lipoprotein, low density lipoprotein, cholesterol and triglycerides were determined using the Cobas C 111 analyzer (F. Hoffmann-La Roche Lts, Switzerland).

Conventional hemodialysis biomarkers were determined by Sonora Quest including serum albumin, serum potassium, and hemoglobin. Thoracic cavity fluid accumulation was obtained by bioimpedance (ZOE® fluid monitor, Noninvasive Medical Technologies, Inc., Las Vegas, NV, http://nmtinc.org/products_zoe.html). Blood pressure was measured at dialysis commencement by the RN or PCT.

Statistical Analysis

Data are reported as mean values ± standard deviation (mean ± SD). For cross-sectional data at baseline, comparisons between groups was performed using a Univariate Analysis. Raw data are reported for each month. Two-way repeated measures ANOVA was used to examine changes over time and between groups at months 0 and 2 and months 0 and 3. In addition, intention-to-treat analysis was performed for those who participated in the study for only 2 months. Normality was assessed and data transformed prior to analyses if necessary. A $p \leq 0.05$ was considered statistically significant. All analyses were performed using PASW (version 19, Chicago, IL).

Chapter 4

RESULTS

Baseline Data

Thirty one participants signed the consent form on visit 1. One participant revealed she was a smoker after the consent form was signed and was removed from the study. Thirty participants were randomized to receive Brazil nuts, selenium pills, or gummy bears (placebo). Before study commencement, 2 participants withdrew from the study stating they no longer wanted to participate after speaking with family members. One patient died after the consent was signed but before the study started. Therefore, 27 participants initiated the study (61.1±17.5y, 14M, 13F). Of the twenty seven participants that started the study, 3 were Native American (11.1%), 5 were Hispanic (18.5%), 5 were African American (18.5%), 12 were Caucasian (44.4%), and 2 were Asian (7.4%). Table 1 shows the baseline characteristics of subjects by group. There were no significant differences between groups.

Table 1. Baseline characteristics of participants within each group[1]

Characteristics	Nut	Pill	Placebo	p value[2]
Gender				
M/F	5/4	4/5	5/4	0.862*
Age (y)	57.1 ± 20.2	64.2 ± 16.8	62.0 ± 16.5	0.694
BMI	28.7 ± 5.7	30.0 ± 7.5	30.7 ± 5.7	0.808
Time on Dialysis (months)	35.0 ± 35.1	29.1 ± 13.4	40.3 ± 26.0	0.672

[1]Data presented as mean ± SD. BMI, body mass index.
[2]p value represents one-way ANOVA (*p value represents chi square analysis)

A total of 9 dropouts were recorded (6M, 3F) during the study. The nut, pill and gummy group lost 5, 1, and 3 participants, respectively. Participants dropped out for the following reasons: complained of itching (n=1), lost to follow up (n=1), received kidney transplant (n=1), refused to continue participation (n=3), thought the gummy bears were too hard (n=1), developed melanoma (n=1), and left the country to take care of family (n=1). One participant in the pill group started the treatment one month late and therefore completed only 2 months of the study. Thus, 17 participants completed the study in its entirety. There was no difference in age, body mass index (BMI), and time on dialysis in participants who completed the study and those that did not complete the study ($p=0.565$, $p=0.564$, and $p=.250$, respectively). At study commencement, each participant was given a four-month calendar and instructed to place an "X" on days they consumed the food or pill. Compliance data were obtained for the 17 participants that completed the study. Of the study's 98 days, the mean days compliant was 93.9 ± 4.6 and did not significantly differ between the three groups ($p=0.719$).

After the study was initiated, 50g of Brazil nuts and 50g of the selenium pills were sent to an independent laboratory (Midwest Laboratories, Omaha, NE) for selenium analyses. These analyses indicated that two Brazil nuts (the daily study dosage) contained 1µg of selenium, a value much below that listed on the National Institutes of Health's dietary supplement page (181µg/2 nuts). The analyses for the selenium tablets indicated that one tablet (the daily study dosage) contained 266µg, a value higher than the label claim, 200µg/1 tablet. Since the study was designed assuming Brazil nuts were an excellent source of selenium and similar to the amount of selenium in the selenium pill, the nut arm of the study was, in essence, a placebo group. Initial analyses confirmed this

as the change in the selenium-dependent marker, plasma glutathione peroxidase, was only noted for the pill group. Hence, the gummy bear and Brazil nut arms of the study were collapsed, and the data hereafter are reported for the pill group (n=9) and the combined 'placebo' group (n=18) only. There remained no significant differences between groups for age, BMI, and time on dialysis (p=0.524, p=0.922, and p=0.427) between the placebo and pill groups. Also, since 4 of the 9 participants that withdrew from the trial did so in the final month of the study, the decision was made to analyze data collected in two ways: at baseline, month 1, and month 2 only, and intention-to-treat analysis for those participants that completed 2 months of the study and were lost during the last month of the study (n=21: 12 placebo and 9 pill).

Antioxidant Status Outcomes

No significant differences were noted for plasma total antioxidant capacity (TAC), vitamin C (VitC), and RBC glutathione peroxidase (GSH-Px) over time or between the placebo (n=12) and pill groups (n=9). A trend was seen in plasma GSH-Px (p=0.08) for a group x time interaction. More specifically, those receiving the pill experienced an increase in plasma GSH-Px while those in the placebo group experienced a decrease in plasma GSH-Px (p=0.023 for change between month 0 and month 2). This significance remained when day of blood draw was controlled for (p=0.031). Table 2 reports data for the antioxidant outcome measures, and Figure 4 shows the change from month 0 to month 2 for plasma and RBC GSH-Px by group; Figure 5 shows the change in plasma and RBC GSH-Px by group from month 0 to month 2 as well as intention-to-treat analysis from month 0 to month 3. A significant positive correlation (r=0.599) was

observed for Epogen, a synthetic hormone given to stimulate RBC production, and RBC GSH-Px during month 2 ($p<0.01$) only.

Table 2. Antioxidant status outcomes by group over time[1]

Variable	Pill (n=9)	Placebo (n=12)	p value[2]	ITT p value[3]	Reference Range[4]
TAC (mM)					1.0-2.30
month 0	1.73 ± 0.7	1.61 ± 0.8			
month 1	1.59 ± 0.6	1.81 ± 0.7			
month 2	2.21 ± 0.7	2.09 ± 0.6	0.708*		
month 3	1.80 ± 0.7	1.66 ± 0.6		0.896*	
VitC (µg ascorbic acid/ml)					0.50-2.0†
month 0	0.38 ± 0.35	0.26 ± 0.27			
month 1	0.24 ± 0.20	0.24 ± 0.18			
month 2	0.25 ± 0.17	0.28 ± 0.25	0.277‡		
month 3	0.26 ± 0.2	0.27 ± 0.2		0.422‡	
RBC GSH-Px (U/g Hb)					20.0-71.0
month 0	66.8 ± 18.5	72.5 ± 16.2			
month 1	63.5 ± 19.6	73.3 ± 18.0			
month 2	69.2 ± 17.5	70.4 ± 15.1	0.409		
month 3	71.5 ± 16.1	67.7 ± 16.0		0.147	
Plasma GSH-Px (nmol/min/ml)					38.0-51.0
month 0	39.8 ± 7.9	47.6 ± 13.9			
month 1	42.2 ± 9.6	41.8 ± 13.5			
month 2	45.8 ± 11.6	43.7 ± 11.5	0.023		
month 3	41.4 ± 10.9	42.5 ± 11.4		0.193‡	

[1]Data presented as mean ± SD, n = 21. Univariate analysis indicated no differences at baseline. ITT, intention to treat; TAC, total antioxidant capacity; VitC, vitamin C; RBC GSH-Px, red blood cell glutathione peroxidase; Plasma GSH-Px, plasma glutathione peroxidase. Assessing for confounders (gender, age, BMI, and time on dialysis) revealed 2 associations: TAC and time on dialysis ($r=-0.528$, $p=0.014$) and VitC and BMI ($r=-0.550$, $p=0.010$).

[2] p value represents two-way repeated measures ANOVA for the group x time interaction at months 0 and 2.

[3] p value represents two-way repeated measures ANOVA for the group x time interaction at months 0 and 3.

[4]Reference standard not established. Ranges indicative of healthy population in recent literature (†reference standard established).

*Covariate controlled for in analyses.

‡Data not normally distributed hence p value for Mann-Whitney analyses change between 0 and 2 months.

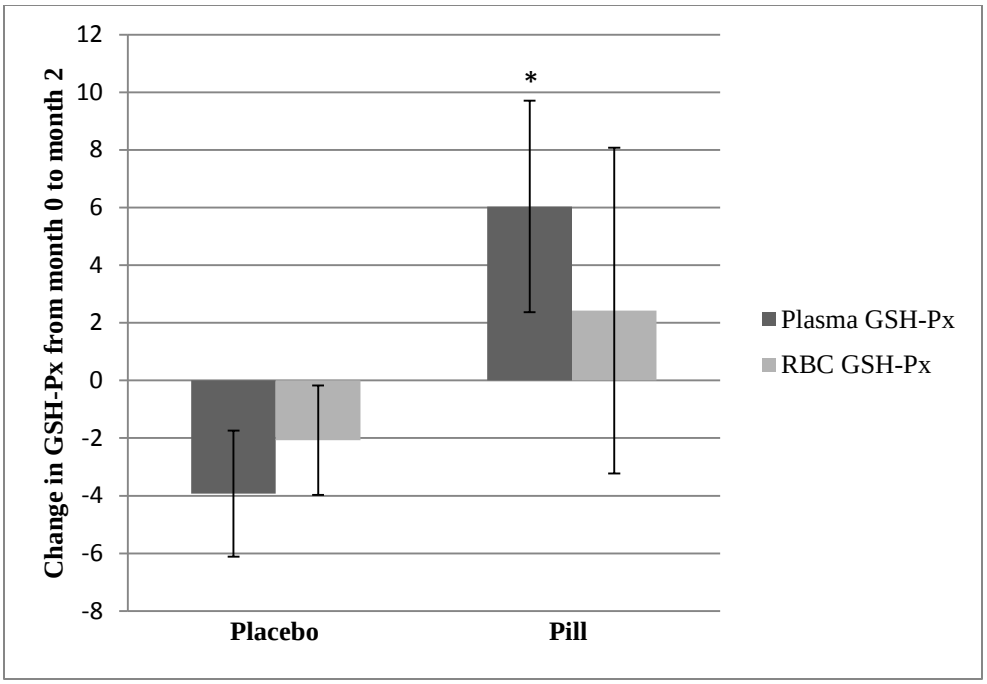


Figure 4. Change in glutathione peroxidase by group (data represents change ± SE)
*p=0.023 between groups

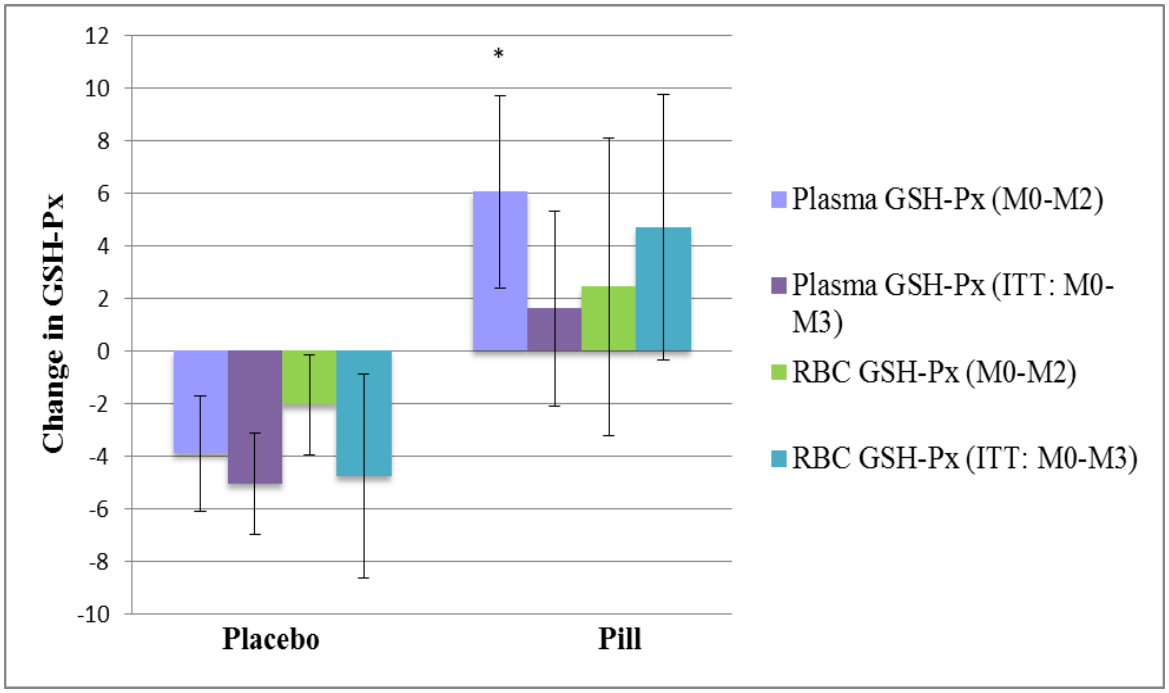


Figure 5. Change in glutathione peroxidase by group with intention-to-treat data analysis (data represents change ± SE)
*p=0.023 between groups

Cardiovascular Disease (CVD) Outcomes

The CVD outcome variables [brain natriuretic peptide (BNP); plasma cholesterol (CHOL), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG); systolic and diastolic blood pressure (BP); and thoracic cavity fluid accumulation (TCFA, lower values indicate more fluid accumulation in the thoracic cavity) values] did not change significantly over time or between groups during the study (Table 3).

Traditional Hemodialysis Markers

There were no statistical differences between groups or over time for serum albumin or serum potassium (Table 4), two traditional biomarkers measured monthly at dialysis units. Additionally, serum potassium remained within normal limits throughout the study despite providing patients with nuts, a higher source of potassium in the food supply. C-reactive protein is a marker for inflammation and is part of the dialysis monthly blood report. Baseline value correlation analysis shows significant negative correlation with HDL ($r=-0.402$, $p=0.037$) suggesting as inflammation worsens, HDL decreases. Alternatively, a significant positive correlation was seen with TG and C-reactive protein ($r=0.576$, $p=0.002$); Table 5 shows baseline correlations with C-reactive protein and plasma HDL, TG and albumin.

Table 3. Cardiovascular disease outcomes by group over time[1]

Variable	Pill (n=9)	Placebo (n=12)	p value[2]	ITT p value[3]	Reference Range[†]
BNP (pg/ml)					<100
month 0	248.5 ± 164.9	258.5 ± 101.3			
month 1	240.1 ± 103.6	230.4 ± 66.0			
month 2	242.3 ± 139.0	300 ± 99.3	0.382[‡]		
month 3	238.7 ± 120.5	240.9 ± 42.1		0.754[‡]	
CHOL (mg/dl)					<200
month 0	124.2 ± 40.1	147.6 ± 21.3			
month 1	143.6 ± 47.2	145.2 ± 33.7			
month 2	140.8 ± 35.3	151.8 ± 31.1	0.216		
month 3	136.5 ± 33.2	147.2 ± 27.9		0.140	
HDL (mg/dl)					>60
month 0	43.8 ± 8.7	47.3 ± 24.8			
month 1	43.5 ± 13.4	45.9 ± 18.3			
month 2	41.8 ± 8.6	50.5 ± 27.4	0.169[‡]		
month 3	41.7 ± 9.2	47.5 ± 24.8		0.554[‡]	
LDL (mg/dl)					<100
month 0	69.1 ± 37.6	77.3 ± 24.0			
month 1	79.6 ± 41.7	74.5 ± 28.1			
month 2	78.8 ± 30.1	75.5 ± 31.2	0.219[‡]		
month 3	73.7 ± 27.8	73.0 ± 30.1		0.219[‡]	

[1]Data presented as mean ± SD, n = 21. Univariate analysis indicated no differences at baseline. ITT, intention to treat; BNP, brain natriuretic peptide; CHOL, cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; BP, blood pressure; TCFA, thoracic cavity fluid accumulation. Assessing for confounders (gender, age, BMI, and time on dialysis) revealed 4 association: HDL and gender (M: 35.5 ± 10.9, F: 57.2 ± 20.5, p=0.006), HDL and BMI (r=-0.458, p=0.037), Diastolic BP and age (r=-0.666, p=0.001), and TCFA and BMI (r=0.436, p=0.048).

[2]p value represents two-way repeated measures ANOVA for the group x time interaction at months 0 and 2.

[3]p value represents two-way repeated measures ANOVA for the group x time interaction at months 0 and 3.

[4]n=19 (placebo = 11, pill = 8)

[†]reference standard established.

[‡]Data not normally distributed hence p value for Mann-Whitney analyses change between 0 and 2 months.

Table 3 continued. Cardiovascular disease outcomes by group over time[1]

Variable	Pill (n=9)	Placebo (n=12)	p value[2]	ITT p value[3]	Reference Range[†]
TG (mg/dl)					<150
month 0	96.2 ± 28.7	149.6 ± 80.0			
month 1	126.8 ± 54.5	157.6 ± 68.6			
month 2	129.0 ± 36.0	159.5 ± 98.8	0.297		
month 3	141.9 ± 45.6	166.1 ± 87.3		0.118	
Systolic BP					<120
month 0	150.8 ± 80.7	143.2 ± 26.6			
month 1	146.6 ± 23.7	134.9 ± 22.7			
month 2	154.8 ± 32.8	138.2 ± 20.5	0.390		
month 3	144.6 ± 26.8	141.6 ± 16.0		0.706	
Diastolic BP					<80
month 0	72.4 ± 8.8	78.6 ± 21.6			
month 1	78.0 ± 24.5	79.1 ± 19.1			
month 2	71.2 ± 14.5	83.1 ± 17.8	0.166[*]		
month 3	73.2 ± 19.5	81.9 ± 18.8		0.493[*]	
TCFA (ohms)[4]					19.0-30.0
month 0	30.0 ± 5.5	30.4 ± 5.2			
month 1	30.8 ± 6.7	30.0 ± 5.4			
month 2	29.0 ± 7.1	29.6 ± 4.8	0.859		
month 3	28.0 ± 6.6	27.2 ± 4.9		0.517[*]	

[1]Data presented as mean ± SD, n = 21. Univariate analysis indicated no differences at baseline. ITT, intention to treat; BNP, brain natriuretic peptide; CHOL, cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; BP, blood pressure; TCFA, thoracic cavity fluid accumulation. Assessing for confounders (gender, age, BMI, and time on dialysis) revealed 4 association: HDL and gender (M: 35.5 ± 10.9, F: 57.2 ± 20.5, p=0.006), HDL and BMI (r=-0.458, p=0.037), Diastolic BP and age (r=-0.666, p=0.001), and TCFA and BMI (r=0.436, p=0.048).

[2]p value represents two-way repeated measures ANOVA for the group x time interaction at months 0 and 2.

[3]p value represents two-way repeated measures ANOVA for the group x time interaction at months 0 and 3.

[4]n=19 (placebo = 11, pill = 8)

[†]reference standard established.

[*]Covariate controlled for in analyses.

[‡]Data not normally distributed hence p value for Mann-Whitney analyses change between 0 and 2 months.

Table 4. Traditional hemodialysis markers by group over time[1]

Variable	Pill (n=9)	Placebo (n=12)	p value[2]	ITT p value[3]	Reference Range[†]
Serum Albumin (g/dL)					>4.0
month 0	3.7 ± 0.5	3.9 ± 0.2			
month 1	3.7 ± 0.6	3.9 ± 0.3			
month 2	3.7 ± 0.6	4.0 ± 0.3	0.153		
month 3	3.7 ± 0.6	3.9 ± 0.2		0.828	
Serum Potassium (mEq/L)					3.5-5.5
month 0	4.4 ± 0.4	4.8 ± 0.9			
month 1	4.7 ± 0.4	4.7 ± 0.7			
month 2	5.0 ± 0.8	5.0 ± 0.6	0.263		
month 3	4.6 ± 0.4	4.9 ± 0.6		0.813	

[1]Data presented as mean ± SD, n = 21. Univariate analysis indicated no differences at baseline. ITT, intention to treat.
[2] p value represents two-way repeated measures ANOVA for the group x time interaction at months 0 and 2.
[3]p value represents two-way repeated measures ANOVA for the group x time interaction at months 0 and 3.
[†]reference standard established.

Table 5. Baseline Correlations of CRP and albumin, TG, and HDL[1]

Variable	Value	correlation (r)	p value[2]
hs-CRP (mg/L)	9.7 ± 10.9		
Serum Albumin (g/dL)	3.9 ± 0.4	-0.338	0.084
Serum TG (mg/dL)	122.8 ± 62.1	0.576	0.002
Serum HDL (mg/dL)	46.7 ± 20.2	-0.402	0.037

[1]Data presented as mean ± SD, n = 27. hs-CRP, high-sensitivity C-reactive protein; TG, triglycerides; HDL, high density lipoprotein
[2] p value represents correlation.

Chapter 5

DISCUSSION

These data demonstrate that selenium supplementation from a pill may be beneficial in improving plasma glutathione peroxidase in maintenance hemodialysis patients. The lack of selenium found in the Brazil nut is unfortunate. The Brazil nut is purported to be the highest source of selenium in the human diet however our independent analysis showed the nuts to be almost void of selenium, despite being grown in Bolivia. The reliability of Brazil nuts as a good source of selenium is questionable and should be used with caution.

Antioxidant Status Outcomes

We demonstrated an increase in plasma GSH-Px, a selenium dependent enzyme synthesized by the kidney,[6] after two months of treatment in the group receiving 266µg of selenium as selenomethionine per day; however, values remained within the normal range and the significance was lost after intention-to-treat analysis for three months was performed. Only two previously published selenium interventions[10, 16] have shown an improvement in plasma GSH-Px in hemodialysis patients, and both used selenite, an inorganic form of selenium. Notably, Saint-Georges et al provided patients with 500µg of selenium orally three times per week after dialysis treatment for 3 months and then reduced the amount to 200µg for the following 3 months. When the amount of selenium was reduced, the levels of plasma GSH-Px did not return to baseline and in fact, remained elevated.[16] Conversely, Richard et al provided patients with 50µg intravenously for 5 weeks and then increased to 100µg for the following 15 weeks. They saw an increase in both plasma and RBC GSH-Px.[10] This suggests a smaller dose of selenium

through intravenous injection may be as potent as a larger dose of selenium given orally. Alternatively, Temple et al and Zachara et al[12, 14, 99] did not show an increase in plasma GSH-Px after selenium supplementation using an inorganic and organic form of selenium, respectively. Notably, Zachara et al[14] stated this was a result of the damaged kidney's inability to synthesize the enzyme. Unlike plasma GSH-Px, most selenium supplementation research in hemodialysis patients has shown supplementation to improve RBC GSH-Px[10, 12, 15, 16, 77, 84] with the exception of one.[99] The studies that showed an increase in the enzyme were at least 2 months in duration, which is enough time to see red blood cell turnover in 2/3 of the body's pool, as the lifespan of the RBC is 3 months. This is a necessary step when evaluating change in RBC GSH-Px as selenium is incorporated into the newly formed RBC during erythropoiesis.[101] In a study lasting 3 month, Zachara et al[12] provided patients either erythropoietin, selenium as selenomethionine (300µg 3x/week), or erythropoietin plus selenium for 3 months. An increase in RBC GSH-Px was seen in both selenium groups (with and without erythropoietin) however not in the erythropoietin group alone. In our study, a significant positive correlation was found between RBC GSH-Px and erythropoietin given in month 2 only ($r=0.599$, $p=0.007$). This coincides with previous research although we did not find a significant difference between groups. It is noteworthy to mention that although we did not see a significant increase in RBC GSH-Px in the pill group, an increase was demonstrated, from 66.8 to 69.2 U/g Hbg. Conversely, the placebo groups experienced a decline in RBC GSH-Px, from 72.5 to 70.4 U/g Hbg. This may suggest a protective effect of the selenium pill compared to the control and a higher dose of selenium may have been needed to see improvement. Lastly, it should be taken into consideration that RBC GSH-

Px is expressed as units/g of hemoglobin and that hemoglobin concentration of dialysis patients is lower than in healthy individuals,[102, 103] possibly inflating the value of RBC GSH-Px of our study population. In fact, the RBC GSH-Px of our study population was within the reference range, and at some points, above the range.

We are the first study to evaluate selenium supplementation on TAC in maintenance hemodialysis patients. Our results showed no difference on TAC by treatment group and over time. Previous research evaluating TAC in HD patients compared to healthy controls varies. TAC has been found to be higher in HD patients compared to healthy controls before dialysis treatment[104-107] but has also been found to be lower.[108] Additionally, TAC has been shown to fluctuate before and after treatment. More specifically, TAC has shown increases[108] or decreases[104, 106] post dialysis treatment compared to pre dialysis treatment. The TAC assay does not differentiate between the various antioxidants in the sample, which include glutathione, ascorbic acid, vitamin E, bilirubin, trolox, bovine serum albumin (BSA) and uric acid. Researchers have suggested the higher TAC levels in HD patients compared to healthy controls can be attributed to their elevated uric acid concentration, as uric acid is excreted by the kidney.[106] While uric acid does not dissipate superoxide, it does require ascorbic acid and thiols to function properly.[109] Alternatively, it has been suggested the elevated TAC levels is not solely because of the uric acid content, but could be a result of the thiols or other substances that have not been identified yet.[107] Despite the reason, the elevation of TAC in HD patients may help protect against the increased oxidative stress these patients undergo.

We did not see a significant change in plasma ascorbic acid by treatment group or over time. When ascorbate, or reduced vitamin C, is oxidized, it is converted to

semidehydroascorbate and further to dehydroascorbate, the oxidized form of vitamin C. The interplay between ascorbate, semidehydroascorbate and dehydroascorbate, and the regeneration of vitamin C, is made possible by both enzyme-dependent and independent pathways, including semidehydroascorbate reductase, an NADH-dependent enzyme, required for the regeneration of ascorbic acid.[110] Vitamin C does not rely solely on GSH systems to regenerate, which may be the cause for the lack of change in vitamin C throughout the study. Alternatively, vitamin E requires ascorbic acid and GSH systems to regenerate in the lipid membrane. The oxidant-antioxidant imbalance in hemodialysis patients is evident by the low plasma vitamin C levels of our study participants. The extracorporeal filtration system used by hemodialysis patients has been suggested to contribute to increased oxidation, further exacerbating the oxidant-antioxidant imbalance. Furthermore, it has been suggested the hemodiafiltration with ultrafiltrate induces less oxidative stress compared to the polysulfone membrane.[111]

Cardiovascular Disease Outcomes

Our results did not show a significant change in brain natriuretic peptide (BNP) between groups or over time. BNP is a 32 amino acid bioactive peptide that is synthesized by the cardiomyocytes and released during hemodynamic stress.[18] It is generally accepted that renal patients present with higher levels of the hormone compared to healthy individuals,[112-114] increasing progressively as the disease worsens[114], due to their inability to clear the hormone as it is normally excreted by the kidneys.[18] In fact, both dialysis dependent and non-dialysis dependent CKD patients present with elevated BNP and plasma levels are correlated with increased left ventricular mass.[115] Our patient population had a higher level of BNP than normal (<100pg/ml) however this may be, in

part, due to the kidneys inability to excrete the hormone. In addition, an increase of BNP is also seen in the elderly.[18] Since the average age of our sample was 61y, this could be another plausible reason for increased levels. The elevated level of BNP is predictive of increased mortality. DeFilippi et al showed CKD patients with a glomerular filtration rate of <60mL/min/1.73m^2 (CKD stage 3-5) and a BNP of $\geq$800ng/l (equivalent to 800pg/ml) with a 260% increased risk of mortality (p=0.004) after adjustment for typical descriptors (i.e. age, sex, etc.) and various comorbidities (i.e. hypertension, heart failure, etc.).[113]

Our study did not show a change in any of the lipid biomarkers, including low density lipoprotein (LDL), high density lipoprotein (HDL), cholesterol (CHOL), triglycerides (TG), systolic blood pressure (sBP), or diastolic BP (dBP). According to the National Cholesterol Education Program Adult Treatment Panel (ATP) III, an optimal lipid panel would consist of the following: LDL < 100mg/dL, HDL > 60mg/dL, and total cholesterol < 200mg/dL.[116] In addition, a triglyceride level of < 150 mg/dL[117] and a blood pressure of <120/80 mm Hg[118] is optimal. Interestingly, the blood pressure treatment guidelines are slightly altered with CKD patients, such that CKD patients require aggressive medication therapy and should be treated with 3 antihypertensive medications if sBP exceeds 130 mg/dL and/or if dBP exceeds 80 mg/dL.[118] The participants in our study had CHOL, LDL, TG, and dBP within the normal limits of healthy adults. The monthly average for each group had an HDL of above 40 mg/dL but was slightly less than optimal (>60 mg/dL). In addition, their sBP was above the recommended 120 mm Hg. This is most likely due to each patient's medication regimen, as 23 of the 27 patients that initiated the study were taking a lipid lowering medication and/or antihypertensive medication. Our patients did, however, present with elevated C-reactive protein (CRP), a

clinical marker of inflammation. Our data is in line with previous research, such that Stage 5 CKD patients present with elevated CRP levels[119], and that in the Stage 5 CKD patient population, CRP is a strong predictor of coronary heart disease.[120]

An overwhelming majority of research has shown clinical effectiveness of statin therapy (i.e.: lowering of low density lipoprotein and total cholesterol) in CKD patients, including those on dialysis.[121-123] Unfortunately, this lipid lowering effect has not always shown beneficial effects in improving stroke, cardiovascular death, and nonfatal myocardial infarction.[124] In fact, a study involving 2776 maintenance hemodialysis patients found a significant decrease in LDL (43% from baseline) after only three months of therapy in those that received 10mg of Rosuvastatin daily. However, a median follow up period of 3.8 years showed statin administration did not affect the primary endpoints of the study: nonfatal stroke, nonfatal myocardial infarction, or cardiovascular death. Additionally, there was no significant effect on all-cause mortality between the placebo and treatment groups.[125]

Limitations

Three major limitations were observed during the course of this study. The first limitation is the debilitating amount of selenium found in the Brazil nuts. As mentioned above, this is unfortunate due to Brazil nuts supposedly being the highest food source of selenium. Due to the variability of selenium in the soil, the amount of selenium in Brazil nuts will continue to vary tremendously and this may be difficult to control for in future research studies. The second limitation of this study was the rather high attrition rate. We experienced a loss of 13 participants after the consent was signed. The highest attrition was seen in the nut group where 6 participants were lost, followed by the placebo group

and pill group who lost 4 and 3 participants, respectively. It is important to note there were no adverse events reported as a result of the study, and the reason for the high attrition was unrelated to the study design or food consumed. The high attrition can be expected with research conducted in a severely diseased population. Sample size calculations described above, showed 12 participants would be required for statistical power in this study. After the study was completed and an accurate standard deviation of the outcome variable and difference of means was determined for our study population, power analysis was calculated at 81 participants per group. The third limitation was limited amount of markers measured. Ideally, markers of lipid peroxidation and troponin T, a marker associated with cardiovascular death and heart failure in the general population and the CKD population, [126] would have been measured during this study to provide a more comprehensive picture regarding the effect of selenium supplementation on the oxidant-antioxidant imbalance. In addition, measuring vitamin E would have also been beneficial in determining lipid membrane stability and vitamin C usage in its regeneration. Due to financial constraints, these markers were not able to be analyzed.

Future Research

Future research evaluating selenium supplementation in hemodialysis patients should be cautious using a food source, specifically when using Brazil nuts. In fact, to ensure that patients are receiving the purported amount of selenium, a supplement should be used. This study found an increase in plasma GSH-Px using an oral supplement of organic selenium as selenomethionine. It would be interesting to compare and contrast the effects of an organic form of selenium to an inorganic form of selenium on plasma GSH-Px. Furthermore, future research should provide a cocktail of antioxidants. When

humans consume food, they consume a plethora of nutrients, not a single nutrient. Because the antioxidant system relies heavily on vitamin C, noting the effects of selenium and vitamin C supplementation in maintenance hemodialysis patients would be useful in determining its effect on overall antioxidant status. Our study measured BNP as a marker for heart failure. Literature has shown BNP's precursor, N-terminal fragment BNP (NT-pro-BNP), has a longer half-life and therefore may be a more accurate indicator of cardiac stress.[18] Lastly, there is void in the literature evaluating selenium supplementation in peritoneal dialysis patients. In fact, there are no published trials to date. Future research should evaluate the effect of selenium and vitamin C supplementation in peritoneal dialysis patients to improve antioxidant status.

Conclusion

Results from this study suggested 266µg/day of selenium as selenomethionine from a tablet consumed for three months increase plasma GSH-Px in maintenance hemodialysis patients. In addition, the low vitamin C status in conjunction with selenium supplementation may have the potential to improve antioxidant status in hemodialysis patients however more research is warranted.

REFERENCES

1. U S renal data system, USRDS 2011 annual data report: Atlas of ChronicKidney disease and end-stage renal disease in the united states,national institutes of health, national institute of diabetes and Digestive and kidney diseases, Bethesda, MD, 2011.

2. Coombes JS, Fassett RG. Antioxidant therapy in hemodialysis patients: A systematic review. *Kidney Int*. 2012;81(3):233-246. doi: 10.1038/ki.2011.341.

3. Loughrey C, Young I, Lightbody J, McMaster D, McNamee P, Trimble E. Oxidative stress in hemodialysis. *Q J Med*. 1994;87(11):679-683.

4. Toborek M, Wasik T, Drozdz M, Klin M, Magnerwrobel K, Kopiecznagrzebieniak E. Effect of hemodialysis on lipid-peroxidation and antioxidant system in patients with chronic-renal-failure. *Metabolism-Clinical and Experimental*. 1992;41(11):1229-1232. doi: 10.1016/0026-0495(92)90014-2.

5. Rico M, Puchades M, Ramon R, Saez G, Tormos M, Miguel A. Effect of oxidative stress in patients with chronic renal failure. *Nefrologia*. 2006;26(2):218-225.

6. Avissar N, Ornt D, Yagil Y, et al. Human kidney proximal tubules are the main source of plasma glutathione-peroxidase. *Am J Physiol*. 1994;266(2):C367-C375.

7. Behne D, Kyriakopoulos A. Mammalian selenium-containing proteins. *Annu Rev Nutr*. 2001;21:453-473. doi: 10.1146/annurev.nutr.21.1.453.

8. Chu F, Esworthy R, Doroshow J, Doan K, Liu X. Expression of plasma glutathione-peroxidase in human liver in addition to kidney, heart, lung, and breast in humans and rodents. *Blood*. 1992;79(12):3233-3238.

9. Yoshimura S, Suemizu H, Nomoto Y, et al. Plasma glutathione peroxidase deficiency caused by renal dysfunction. *Nephron*. 1996;73(2):207-211.

10. Richard M, Ducros V, Foret M, et al. Reversal of selenium and zinc deficiencies in chronic-hemodialysis patients by intravenous-sodium selenite and zinc gluconate supplementation - time-course of glutathione-peroxidase repletion and lipid-peroxidation decrease. *Biol Trace Elem Res*. 1993;39(2-3):149-159. doi: 10.1007/BF02783185.

11. Pakfetrat M, Malekmakan L, Hasheminasab M. Diminished selenium levels in hemodialysis and continuous ambulatory peritoneal dialysis patients. *Biol Trace Elem Res*. 2010;137(3):335-339. doi: 10.1007/s12011-009-8588-2.

12. Zachara B, Adamowicz A, Trafikowska U, Trafikowska A, Manitius J, Nartowicz E. Selenium and glutathione levels, and glutathione peroxidase activities in blood components of uremic patients on hemodialysis supplemented with selenium and

treated with erythropoietin. *Journal of Trace Elements in Medicine and Biology*. 2001;15(4):201-208. doi: 10.1016/S0946-672X(01)80034-1.

13. Temple K, Smith A, Cockram D. Selenate supplementation increases plasma selenium (se) in hemodialysis (HD) patients. *Faseb Journal*. 1997;11(3):2081-2081.

14. Zachara BA, Gromadzinska J, Zbrog Z, et al. Selenium supplementation to chronic kidney disease patients on hemodialysis does not induce the synthesis of plasma glutathione peroxidase RID G-4913-2010 RID F-6006-2010 RID A-5917-2012 RID F-6005-2010. *Acta Biochim Pol*. 2009;56(1):183-187.

15. Stockler-Pinto MB, Mafra D, Farage NE, Boaventura GT, Cozzolino SMF. Effect of brazil nut supplementation on the blood levels of selenium and glutathione peroxidase in hemodialysis patients. *Nutrition*. 2010;26(11-12):1065-1069. doi: 10.1016/j.nut.2009.08.006.

16. Saint-Georges M, Bonnefont D, Bourely B, et al. Correction of selenium deficiency in hemodialyzed patients. *Kidney Int*. 1989;36:S274-S277.

17. Thomson CD, Chisholm A, McLachlan SK, Campbell JM. Brazil nuts: An effective way to improve selenium status. *Am J Clin Nutr*. 2008;87(2):379-384.

18. Braunwald E. Biomarkers in heart failure. *N Engl J Med*. 2008;358(20):2148-2159. doi: 10.1056/NEJMra0800239.

19. Descamps-Latscha B, Drueke T, Witko-Sarsat V. Dialysis-induced oxidative stress: Biological aspects, clinical consequences, and therapy. *Semin Dial*. 2001;14(3):193-199. doi: 10.1046/j.1525-139X.2001.00052.x.

20. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: Critical view and experimental data. *Free Radical Biology and Medicine*. 2000;29(11):1106-1114. doi: 10.1016/S0891-5849(00)00394-4.

21. Bonomini F, Tengattini S, Fabiano A, Bianchi R, Rezzani R. Atherosclerosis and oxidative stress. *Histol Histopathol*. 2008;23(3):381-390.

22. Locatelli F, Canaud B, Eckardt K, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: An emerging threat to patient outcome. *Nephrology Dialysis Transplantation*. 2003;18(7):1272-1280. doi: 10.1093/ndt/gfg074.

23. Pryor W. Oxyradicals and related species - their formation, lifetimes, and reactions. *Annu Rev Physiol*. 1986;48:657-667. doi: 10.1146/annurev.physiol.48.1.657.

24. Leiva E, Mujica V, Sepulveda P, et al. High levels of iron status and oxidative stress in patients with metabolic syndrome. *Biol Trace Elem Res*. 2013;151(1):1-8. doi: 10.1007/s12011-012-9525-3.

25. Gropper SAS, Smith JL, Groff JL. Selenium. In: *Advanced Nutrition and Human Metabolism.* Wadsworth Cengage Learning; 2009:506.

26. Johnston C, Meyer C, Srilakshmi J. Vitamin-C elevates red-blood-cell glutathione in healthy-adults. *Am J Clin Nutr*. 1993;58(1):103-105.

27. Nicholson J, Wolmarans M, Park G. The role of albumin in critical illness. *Br J Anaesth*. 2000;85(4):599-610. doi: 10.1093/bja/85.4.599.

28. van Meeteren M, Teunissen C, Dijkstra C, van Tol E. Antioxidants and polyunsaturated fatty acids in multiple sclerosis. *Eur J Clin Nutr*. 2005;59(12):1347-1361. doi: 10.1038/sj.ejcn.1602255.

29. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. The National Academies Press; 2000.

30. Rayman MP, Infante HG, Sargent M. Food-chain selenium and human health: Spotlight on speciation. *Br J Nutr*. 2008;100(2):238-253. doi: 10.1017/S0007114508922522.

31. Monsen E. Dietary reference intakes fop the antioxidant nutrients: Vitamin C, vitamin E, selenium, and carotenoids. *J Am Diet Assoc*. 2000;100(6):637-640. doi: 10.1016/S0002-8223(00)00189-9.

32. Shils ME, Shike M. *Modern Nutrition in Health and Disease.* Lippincott Williams & Wilkins; 2006.

33. Thomson C, Robinson M. Urinary and fecal excretions and absorption of a large supplement of selenium - superiority of selenate over selenite. *Am J Clin Nutr*. 1986;44(5):659-663.

34. Daniels L. Selenium metabolism and bioavailability. *Biol Trace Elem Res*. 1996;54(3):185-199. doi: 10.1007/BF02784430.

35. Behne D, Kyriakopoulos A, Scheid S, Gessner H. Effects of chemical form and dosage on the incorporation of selenium into tissue proteins in rats. *J Nutr*. 1991;121(6):806-814.

36. Behne D, Kyriakopoulos A. Mammalian selenium-containing proteins. *Annu Rev Nutr*. 2001;21:453-473. doi: 10.1146/annurev.nutr.21.1.453.

37. Combs G, Combs S. The nutritional biochemistry of selenium. *Annu Rev Nutr*. 1984;4:257-280. doi: 10.1146/annurev.nutr.4.1.257.

38. Waschulewski I, Sunde R. Effect of dietary methionine on tissue selenium and glutathione-peroxidase (Ec1.11.1.9) activity in rats given selenomethionine. *Br J Nutr*. 1988;60(1):57-68. doi: 10.1079/BJN19880076.

39. Burk R, Brown D, Scaief C, Seely R. Influence of dietary and injected selenium on whole-body retention, route of excretion, and tissue retention of se-75o32- in rat. *J Nutr*. 1972;102(8):1049-&.

40. MILLS G. Hemoglobin catabolism .1. glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. *J Biol Chem*. 1957;229(1):189-197.

41. Rotruck J, Hoekstra W, Ganther H, Pope A. Prevention of oxidative damage to rat erythrocytes by dietary selenium. *J Nutr*. 1972;102(5):689-&.

42. Tappel M, Ccaudiere J, Tappel A. Glutathione-peroxidase activities of animal-tissues. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*. 1982;73(4):945-949. doi: 10.1016/0305-0491(82)90341-8.

43. Gamble S, Wiseman A, Goldfarb P. Selenium-dependent glutathione peroxidase and other selenoproteins: Their synthesis and biochemical roles. *Journal of Chemical Technology and Biotechnology*. 1997;68(2):123-134. doi: 10.1002/(SICI)1097-4660(199702)68:2<123::AID-JCTB641>3.0.CO;2-O.

44. Johnston C, Meyer C, Srilakshmi J. Vitamin-C elevates red-blood-cell glutathione in healthy-adults. *Am J Clin Nutr*. 1993;58(1):103-105.

45. Sokol R. Vitamin-E-deficiency and neurologic disease. *Annu Rev Nutr*. 1988;8:351-373. doi: 10.1146/annurev.nutr.8.1.351.

46. Pimentel L. Scurvy: Historical review and current diagnostic approach. *Am J Emerg Med*. 2003;21(4):328-332. doi: 10.1016/S0735-6757(03)00083-4.

47. Larsen P, Berry M. Nutritional and hormonal-regulation of thyroid-hormone deiodinases. *Annu Rev Nutr*. 1995;15:323-352. doi: 10.1146/annurev.nutr.15.1.323.

48. Patching S, Gardiner P. Recent developments in selenium metabolism and chemical speciation: A review. *J Trace Elem Med Biol*. 1999;13(4):193-214.

49. Mostert V. Selenoprotein P: Properties, functions, and regulation. *Arch Biochem Biophys*. 2000;376(2):433-438. doi: 10.1006/abbi.2000.1735.

50. Mezes M, Balogh K. Prooxidant mechanisms of selenium toxicity - a review. *Acta Biologica Szegediensis*. 2009;53 (Suppl 1):15-18.

51. Surai PF. *Selenium in Nutrition and Health.* Paul & Company Pub Consortium; 2006.

52. Yan L, Spallholz J. Generation of reactive oxygen species from the reaction of selenium-compounds with thiols and mammary-tumor cells. *Biochem Pharmacol*. 1993;45(2):429-437. doi: 10.1016/0006-2952(93)90080-G.

53. Garberg P, Stahl A, Warholm M, Hogberg J. Studies of the role of dna fragmentation in selenium toxicity. *Biochem Pharmacol*. 1988;37(18):3401-3406. doi: 10.1016/0006-2952(88)90688-0.

54. Ganther H. Selenium metabolism, selenoproteins and mechanisms of cancer prevention: Complexities with thioredoxin reductase. *Carcinogenesis*. 1999;20(9):1657-1666. doi: 10.1093/carcin/20.9.1657.

55. Kelly S, Havrilla C, Brady T, Abramo K, Levin E. Oxidative stress in toxicology: Established mammalian and emerging piscine model systems. *Environ Health Perspect*. 1998;106(7):375-384. doi: 10.2307/3434064.

56. Brozmanova J, Manikova D, Vlckova V, Chovanec M. Selenium: A double-edged sword for defense and offence in cancer. *Arch Toxicol*. 2010;84(12):919-938. doi: 10.1007/s00204-010-0595-8.

57. Redman C, Scott J, Baines A, et al. Inhibitory effect of selenomethionine on the growth of three selected human tumor cell lines. *Cancer Lett*. 1998;125(1-2):103-110. doi: 10.1016/S0304-3835(97)00497-7.

58. Jariwalla RJ, Gangapurkar B, Nakamura D. Differential sensitivity of various human tumour-derived cell types to apoptosis by organic derivatives of selenium. *Br J Nutr*. 2009;101(2):182-189. doi: 10.1017/S0007114508998305.

59. Tanaka T, Kohno H, Murakami M, Kagami S, El-Bayoumy K. Suppressing effects of dietary supplementation of the organoselenium 1,4-phenylenebis(methylene)selenocyanate and the citrus antioxidant auraptene on lung metastasis of melanoma cells in mice. *Cancer Res*. 2000;60(14):3713-3716.

60. Zhang E, Wang H, Yan X, Zhang L. Comparison of short-term toxicity between nano-se and selenite in mice. *Life Sci*. 2005;76(10):1099-1109. doi: 10.1016/j.lfs.2004.08.015.

61. Rayman MP. Selenium and human health. *Lancet*. 2012;379(9822):1256-1268. doi: 10.1016/S0140-6736(11)61452-9.

62. Bock A, Forchhammer K, Heider J, et al. Selenocysteine - the 21st amino-acid. *Mol Microbiol*. 1991;5(3):515-520. doi: 10.1111/j.1365-2958.1991.tb00722.x.

63. Ge K, Yang G. The epidemiology of selenium deficiency in the etiologic study of endemic diseases in china. *Am J Clin Nutr*. 1993;57(2):S259-S263.

64. Yang G. Research on selenium-related problems in human health in china. In: Combs G, Spallholz J, Levander O, Oldfield J, eds. *Selenium in Biology in Medicine. Proceedings of the Third International Symposium on Selenium (Part A).* Van Hostrand Reinhold Company; 1987:9-32.

65. Yang G, Ge K, Chen J, Chen X.
Selenium-related endemic diseases and the daily selenium requirement of humans. *World Rev Nutr Diet*. 1988;55:98-152.

66. [Anonymous]. Epidemiologic studies on the etiologic relationship of selenium and keshan disease. *Chin Med J*. 1979;92(7):477-482.

67. Ren L, Li X, Li G, Zhao Z, Sun B, Sun F.
Coxsackievirus B_3 infection and its mutation in keshan disease
. *World J Gastroenterol*. 2004;10(22):3299-3302.

68. Kok F, Hofman A, Witteman J, et al. Decreased selenium levels in acute myocardial-infarction. *JAMA-J Am Med Assoc*. 1989;261(8):1161-1164. doi: 10.1001/jama.261.8.1161.

69. Salonen J, Alfthan G, Huttunen J, Pikkarainen J, Puska P. Association between cardiovascular death and myocardial-infarction and serum selenium in a matched-pair longitudinal-study. *Lancet*. 1982;2(8291):175-179.

70. Wei W, Abnet C, Qiao Y, et al. Prospective study of serum selenium concentrations and esophageal and gastric cardia cancer, heart disease, stroke, and total death. *Am J Clin Nutr*. 2004;79(1):80-85.

71. Stranges S, Marshall J, Trevisan M, et al. Effects of selenium supplementation on cardiovascular disease incidence and mortality: Secondary analyses in a randomized clinical trial. *Am J Epidemiol*. 2006;163(8):694-699. doi: 10.1093/aje/kwj097.

72. Flores-Mateo G, Navas-Acien A, Pastor-Barriuso R, Guallar E. Selenium and coronary heart disease: A meta-analysis. *Am J Clin Nutr*. 2006;84(4):762-773.

73. Maggi E, Bellazzi R, Falaschi F, et al. Enhanced ldl oxidation in uremic patients - an additional mechanism for accelerated atherosclerosis. *Kidney Int*. 1994;45(3):876-883. doi: 10.1038/ki.1994.115.

74. Jackson P, Loughrey C, Lightbody J, McNamee P, Young I. Effect of hemodialysis on total antioxidant capacity and serum antioxidants in patients with chronic-renal-failure. *Clin Chem*. 1995;41(8):1135-1138.

75. Yavuz O, Bicik Z, Cinar Y, Guney Y, Guler S. The effect of different dialysis membranes on oxidative stress and selenium status. *Clinica Chimica Acta*. 2004;346(2):153-160. doi: 10.1016/j.cccn.2004.03.025.

76. Morena M, Cristol J, Bosc J, et al. Convective and diffusive losses of vitamin C during haemodiafiltration session: A contributive factor to oxidative stress in haemodialysis patients. *Nephrology Dialysis Transplantation*. 2002;17(3):422-427. doi: 10.1093/ndt/17.3.422.

77. Koenig J, Fischer M, Bulant E, Tiran B, Elmadfa I, Druml W. Antioxidant status in patients on chronic hemodialysis therapy: Impact of parenteral selenium supplementation. *Wien Klin Wochenschr*. 1997;109(1):13-19.

78. Canaud B, Cristol J, Morena M, Leray-Moragues H, Bosc J, Vaussenat F. Imbalance of oxidants and antioxidants in haemodialysis patients. *Blood Purif*. 1999;17(2-3):99-106. doi: 10.1159/000014381.

79. Cristol J, Canaud B, Rabesandratana H, Gaillard I, Serre A, Mion C. Enhancement of reactive oxygen species production and cell-surface markers expression due to hemodialysis. *Nephrology Dialysis Transplantation*. 1994;9(4):389-394.

80. Knudsen P, Shaldon S, Floege J, Koch M. Hemodialysis-related induction of beta-2-microglobulin synthesis and release by mononuclear phagocytes. *Int J Artif Organs*. 1990;13(2):73-76.

81. Peuchant E, Carbonneau M, Dubourg L, et al. Lipoperoxidation in plasma and red-blood-cells of patients undergoing hemodialysis - vitamin-A, vitamin-E, and iron status. *Free Radical Biology and Medicine*. 1994;16(3):339-346. doi: 10.1016/0891-5849(94)90035-3.

82. Sommerburg O, Grune T, Hampl H, et al. Does long-term treatment of renal anaemia with recombinant erythropoietin influence oxidative stress in haemodialysed patients?. *Nephrology Dialysis Transplantation*. 1998;13(10):2583-2587. doi: 10.1093/ndt/13.10.2583.

83. Hill K, Burk R, Lane J. Effect of selenium depletion and repletion on plasma glutathione and glutathione-dependent enzymes in the rat. *J Nutr*. 1987;117(1):99-104.

84. Bonomini M, Forster S, Derisio F, et al. Effects of selenium supplementation on immune parameters in chronic uremic patients on hemodialysis. *Nephrol Dial Transplant*. 1995;10(9):1654-1661.

85. Foote J, Hinks L, Lloyd B. Reduced plasma and white blood-cell selenium levels in hemodialysis-patients. *Clinica Chimica Acta*. 1987;164(3):323-328. doi: 10.1016/0009-8981(87)90307-X.

86. Antos M, Jerenstrujic B, Romic Z, Matanovic B, Gudelgreguric J. Serum selenium levels in patients on hemodialysis. *Trace Elements in Medicine*. 1993;10(4):173-176.

87. Bogye G, Tompos G, Alfthan G. Selenium depletion in hemodialysis patients treated with polysulfone membranes. *Nephron*. 2000;84(2):119-123. doi: 10.1159/000045558.

88. Marti del Moral L, Agil A, Navarro-Alarcon M, Lopez-Ga de la Serrana H, Palomares-Bayo M, Jesus Oliveras-Lopez M. Altered serum selenium and uric acid

levels and dyslipidemia in hemodialysis patients could be associated with enhanced cardiovascular risk. *Biol Trace Elem Res*. 2011;144(1-3):496-503. doi: 10.1007/s12011-011-9152-4.

89. Apostolidis N, Panoussopoulos D, Stamou K, et al. Selenium metabolism in patients on continuous ambulatory peritoneal dialysis. *Peritoneal Dialysis International*. 2002;22(3):400-404.

90. Charney D, Gouge S, Charney P, Shippee R, Merrill G. Selenium (se) deficiency in patients on chronic-hemodialysis (hd). *Kidney Int*. 1990;37(1):290-290.

91. Dworkin B, Weseley S, Rosenthal W, Schwartz E, Weiss L. Diminished blood selenium levels in renal-failure patients on dialysis - correlations with nutritional-status. *Am J Med Sci*. 1987;293(1):6-12.

92. Zachara BA, Gromadzinska J, Wasowicz W, Zbrog Z. Red blood cell and plasma glutathione peroxidase activities and selenium concentration in patients with chronic kidney disease: A review RID G-4913-2010 RID F-6006-2010. *Acta Biochim Pol*. 2006;53(4):663-677.

93. Sriram K, Abraham G. Loss of zinc and selenium does not occur through peritoneal dialysis. *Nutrition*. 2000;16(11-12):1047-1051. doi: 10.1016/S0899-9007(00)00429-9.

94. Diskin C. Does abnormal trace element metabolism contribute to dialysis patient morbidity?. *Semin Dial*. 1999;12(1):18-20. doi: 10.1046/j.1525-139X.1999.12103.x.

95. Bonomini M, Mujais S, Ivanovich P, Klinkmann H. Selenium in uremia - culprit or bystander. *Nephron*. 1992;60(4):385-389. doi: 10.1159/000186796.

96. Zagrodzki P, Barton H, Walas S, et al. Selenium status indices, laboratory data, and selected biochemical parameters in end-stage renal disease patients. *Biol Trace Elem Res*. 2007;116(1):29-41. doi: 10.1007/BF02685916.

97. Sarnak M, Levey A, Schoolwerth A, et al. Kidney disease as a risk factor for development of cardiovascular disease - A statement from the american heart association councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. *Circulation*. 2003;108(17):2154-2169. doi: 10.1161/01.CIR.0000095676.90936.80.

98. Capusa C, Stoian I, Rus E, Lixandru D, Barbulescu C, Mircescu G. Does dialysis modality influence the oxidative stress of uremic patients?. *Kidney Blood Press Res*. 2012;35(4):220-225. doi: 10.1159/000331560.

99. Temple K, Smith A, Cockram D. Selenate-supplemented nutritional formula increases plasma selenium in hemodialysis patients. *J Ren Nutr*. 2000;10:16-23.

100. [Anonymous]. National kidney foundation releases K/DOQI nutrition guidelines for dialysis patients. *Dialysis & Transplantation*. 2000;29(8):264-264.

101. Perona G, Guidi G, Piga A, Cellerino R, Menna R, Zatti M. Invivo and invitro variations of human erythrocyte glutathione peroxidase-activity as result of cells aging, selenium availability and peroxide activation. *Br J Haematol*. 1978;39(3):399-408. doi: 10.1111/j.1365-2141.1978.tb01111.x.

102. Collins A. Influence of target hemoglobin in dialysis patients on morbidity and mortality. *Kidney Int*. 2002;61:S44-S48.

103. Eckardt K. Target hemoglobin in patients with renal failure. *Nephron*. 2001;89(2):135-143. doi: 10.1159/000046060.

104. Montazerifar F, Hashemi M, Karajibani M, Dikshit M. Hemodialysis alters lipid profiles, total antioxidant capacity, and vitamins A, E, and C concentrations in humans. *Journal of Medicinal Food*. 2010;13(6):1490-1493. doi: 10.1089/jmf.2010.1074.

105. Ahmadpoor P, Eftekhar E, Nourooz-Zadeh J, Servat H, Makhdoomi K, Ghafari A. Glutathione, glutathione-related enzymes, and total antioxidant capacity in patients on maintenance dialysis. *Iranian Journal of Kidney Diseases*. 2009;3(1):22-27.

106. Jackson P, Loughrey C, Lightbody J, McNamee P, Young I. Effect of hemodialysis on total antioxidant capacity and serum antioxidants in patients with chronic-renal-failure. *Clin Chem*. 1995;41(8):1135-1138.

107. Mircescu G, Capusa C, Stoian I, Vargolici B, Barbulescu C, Ursea N. Global assessment of serum antioxidant status in hemodialysis patients. *J Nephrol*. 2005;18(5):599-605.

108. Coaccioli S, Standoli ML, Biondi R, et al. Assessment of the oxidative stress markers in patients with chronic renal insufficiency undergoing dialysis treatment. *Clinica Terapeutica*. 2010;161(5):441-444.

109. Sautin YY, Johnson RJ. Uric acid: The oxidant-antioxidant paradox. *Nucleosides Nucleotides & Nucleic Acids*. 2008;27(6-7):608-619. doi: 10.1080/15257770802138558.

110. Wells W, W., Jung C. Regeneration of vitamin C. In: Packer L, Fuchs J, eds. *Vitamin C in Health and Disease.* New York: Marcel Dekker, Inc.; 1997:109-121.

111. Gonzalez-Diez B, Cavia M, Torres G, Abaigar P, Muniz P. Effect of a hemodiafiltration session with on-line regeneration of the ultrafiltrate on oxidative stress. *Blood Purif*. 2008;26(6):505-510. doi: 10.1159/000163848.

112. Vickery S, Price C, John R, et al. B-type natriuretic peptide (BNP) and amino-terminal proBNP in patients with CKD: Relationship to renal function and left ventricular hypertrophy. *American Journal of Kidney Diseases*. 2005;46(4):610-620. doi: 10.1053/j.ajkd.2005.06.017.

113. Defilippi CR, Seliger SL, Maynard S, Christenson RH. Impact of renal disease on natriuretic peptide testing for diagnosing decompensated heart failure and predicting mortality. *Clin Chem*. 2007;53(8):1511-1519. doi: 10.1373/clinchem.2006.084533.

114. Li X, Yang X, Sun Q, Chen X, Li Y. Brain natriuretic peptide and copeptin levels are associated with cardiovascular disease in patients with chronic kidney disease. *Chin Med J (Engl)*. 2013;126(5):823-7.

115. Locatelli F, Vigano S. Are natriuretic peptides a reliable marker for mortality in ESRD patients?. *Nephrology Dialysis Transplantation*. 2010;25(2):347-349. doi: 10.1093/ndt/gfp606.

116. Cleeman J, Grundy S, Becker D, et al. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *Jama-Journal of the American Medical Association*. 2001;285(19):2486-2497.

117. Miller M, Stone NJ, Ballantyne C, et al. Triglycerides and cardiovascular disease A scientific statement from the american heart association. *Circulation*. 2011;123(20):2292-2333. doi: 10.1161/CIR.0b013e3182160726.

118. Chobanian A, Bakris G, Black H, et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure - the JNC 7 report. *JAMA-J Am Med Assoc*. 2003;289(19):2560-2572. doi: 10.1001/jama.289.19.2560.

119. Arici M, Walls J. End-stage renal disease, atherosclerosis, and cardiovascular mortality: Is C-reactive protein the missing link?. *Kidney Int*. 2001;59(2):407-414. doi: 10.1046/j.1523-1755.2001.059002407.x.

120. Danesh J, Wheeler JG, Hirschfield GM, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med*. 2004;350(14):1387-1397. doi: 10.1056/NEJMoa032804.

121. Hou W, Lv J, Perkovic V, et al. Effect of statin therapy on cardiovascular and renal outcomes in patients with chronic kidney disease: A systematic review and meta-analysis. *Eur Heart J*. 2013 Mar 6;[Epub ahead of print].

122. Makowka A, Dryja P, Chwatko G, Bald E, Nowicki M. Treatment of chronic hemodialysis patients with low-dose fenofibrate effectively reduces plasma lipids and affects plasma redox status. *Lipids in Health and Disease*. 2012;11:47. doi: 10.1186/1476-511X-11-47.

123. Strippoli GFM, Navaneethan SD, Johnson DW, et al. Effects of statins in patients with chronic kidney disease: Meta-analysis and meta-regression of randomised controlled trials. *Br Med J*. 2008;336(7645):645-651. doi: 10.1136/bmj.39472.580984.AE.

124. Wanner C, Krane V, Marz W, et al. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. *N Engl J Med*. 2005;353(3):238-248. doi: 10.1056/NEJMoa043545.

125. Fellstroem BC, Jardine AG, Schmieder RE, et al. Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. *N Engl J Med*. 2009;360(14):1395-1407. doi: 10.1056/NEJMoa0810177.

126. Mishra R, Li Y, Defilippi C, et al. Association of Cardiac Troponin T With left ventricular structure and function in CKD. *Am J Kidney Dis*. 2013 Jan 3;12:01474-6. doi: 10.1053/j.ajkd.2012.11.034.

APPENDIX A

IRB APPROVAL AND CONSENT FORM

ASU Knowledge Enterprise Development

Office of Research Integrity and Assurance

To:	Carol Johnston ABC 132
From:	Carol Johnston, Chair Bioscience Full Board
Date:	08/29/2012
Committee Action:	Approval
IRB Action Date	08/29/2012
Approval Date	08/15/2012
IRB Protocol #	1208008114
Study Title	Selenium Supplementation and Cardiovascular Outcome Markers in Hemodialysis Patients
Expiration Date	08/14/2013

The above-referenced protocol has been APPROVED following Full Board Review by the Institutional Review Board.

This approval does not replace any departmental or other approvals that may be required. It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date noted above. Please allow sufficient time for continued approval. Research activity of any sort may not continue beyond the expiration date without committee approval. Failure to receive approval for continuation before the expiration date will result in the automatic suspension of the approval of this protocol on the expiration date. Information collected following suspension is unapproved research and cannot be reported or published as research data. If you do not wish continued approval, please notify the Committee of the study termination.

Adverse Reactions: If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Bioscience Full Board immediately. If necessary a member of the Committee will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review.

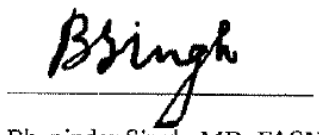
Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Bioscience Full Board. The new procedure is not to be initiated until the IRB approval has been given.

Letter of Cooperation

Southwest Clinical Research Institute (SCRI), LLC looks forward to a cooperative relationship with the School of Nutrition and Health Promotion, Arizona State University, Tempe, AZ for the purposes of conducting a clinical research study among patients with End Stage Renal Disease who are on hemodialysis. The study will be conducted at one or more outpatient hemodialysis centers owned and operated by Southwest Kidney Institute and Davita (JV), as mutually identified by the clinical research team. A Business Associate contract will be created to incorporate HIPAA and Confidentiality related requirements.

The study will need to be IRB approved. Southwest Clinical Research Institute accepts approvals granted by Central and Academic IRBs. Once the protocol has been approved by the ASU IRB, it will be presented to the research committee of SCRI, and the Board of JV for approval.

In case of any questions, please feel free to contact me at (480) 610-6120.



Bhupinder Singh, MD, FASN

Medical Director, Southwest Clinical Research Institute

7/27/2012

Selenium Supplementation and Health Outcomes in Hemodialysis Patients

INTRODUCTON
The purposes of this form are (1) to provide you with information that may affect your decision as to whether or not to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

RESEARCHERS
Dr. Carol Johnston, a Nutrition professor at Arizona State and Elizabeth Sussman, nutrition doctoral student, have requested your participation in a research study.

STUDY PURPOSE
The purpose of the research is to evaluate the effect of selenium supplementation on health parameters in hemodialysis patients, including risk for heart disease and inflammation. Selenium is an important antioxidant in the diet; however, hemodialysis patients are often low in this nutrient due to the dialysis process. In this study, patients will be assigned to ingest either Brazil nuts or dietary supplements daily for 90 days.

DESCRIPTION OF RESEARCH STUDY
Patients from the DaVita-Southwest Kidney Institute dialysis center who are on maintenance hemodialysis (>90 days) with a fistula or graft for hemodialysis access and generally healthy will be invited to participate in this study which will encompass 5 short meetings at the dialysis clinic. To be eligible to participate you must be >18 years of age and a non-smoker who does not take antioxidant supplements and is not allergic to nuts or yeast. As a research participant, you will need to restrict Brazil nuts and certain dietary supplements for 3 months (the length of the study). All study visits will take place prior to the start of your regularly scheduled dialysis treatment. These meetings will take less than 30 minutes. At the first meeting, you will complete a brief health history questionnaire to demonstrate the absence of medical conditions or dietary habits that may impact the study. At all meetings, your weight will be measured and your heart function will be assessed by bioelectrical impedance, a noninvasive procedure that sends a small electrical current through the chest cavity. The 2nd, 3rd, 4th, and 5th meetings will be scheduled at one-month intervals to align with your regularly scheduled blood draw days at the dialysis center. Two additional tubes of blood will be collected on these visits to examine measures related to heart disease risk and inflammation.

You will be assigned to a dietary intervention group (Brazil nuts or supplement) at the 2nd meeting and receive either nuts or supplements to take home. You will mark that you ingested the product (nuts or supplements) each day on a calendar to be posted at home on your refrigerator. Otherwise, you are to follow your normal diet during the study. If you start a diet program or alter your normal diet during the study, please notify the research staff. About 30 dialysis patients will participate in this study. Blood samples will be analyzed for substances associated with selenium status, heart disease risk, and inflammation.

RISKS
The experimental foods are Brazil nuts and yeast. If you are allergic to nuts or yeast, you cannot participate in this trial. You will be asked to immediately report any of the following symptoms to the investigators: skin reactions, such as hives, redness or swelling; itching or tingling in or around the mouth and throat; digestive problems, such as diarrhea, stomach cramps, nausea or vomiting; tightening of the throat; shortness of breath or wheezing; and runny nose. There is a small risk that unknown nut allergy can result in allergic reaction and death. Blood will be collected routinely on your regularly scheduled blood draw day from your fistula or graft.

BENEFITS
This study will provide information regarding the usefulness of additional dietary selenium for improving health parameters in dialysis patients.

NEW INFORMATION
If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators. Plasma from blood samples will be stored for 5 years in freezers in the laboratories of the Nutrition Program at Arizona State University after which time they will be disposed of as biohazard waste.

WITHDRAWAL PRIVILEGE

You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision will not affect your treatment at the DaVita-Southwest Kidney Institute dialysis center in any manner.

COSTS AND PAYMENTS

During the experimental periods, you will be provided with free product (Brazil nuts or supplements). You will also receive $15 gift certificates to Target at visits 3 and 5 ($30 total).

COMPENSATION FOR ILLNESS AND INJURY

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, in the event of harm, injury, or illness arising from this study, neither Arizona State University nor the researchers are able to give you any money, insurance coverage, free medical care, or any compensation for such injury. Major injury is not likely but if necessary, a call to 911 will be placed.

VOLUNTARY CONSENT

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston (carol.johnston@asu.edu or 602-827-2265) or Elizabeth Sussman (esussman@asu.edu).

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Research Compliance Office, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

_____	_____	_____
Subject's Signature	Printed Name	Date
_____	_____	ASU IRB APPROVED
Contact phone number	Email	8/15/12-8/14/13

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

APPENDIX B

QUESTIONNAIRE AND HANDOUT

MEDICAL HISTORY QUESTIONNAIRE ID#_____

Age: _____ Gender: □ Male □ Female Smoker: □Yes □No □Quit

Height _____ft _____in. EDW Weight: _____kg. Waist: _____cm

Ethnicity (circle): Native American African-American non-Hispanic White Hispanic Asian

1. Do you take any medications regularly? Please list what kind and how frequently:

Medication Dosage Frequency

_____
_____
_____
_____
_____
_____
_____
_____

2. Do you currently take supplements (vitamins, minerals, herbs, etc.)? Y N
If yes, what supplements and how often?

_____
_____
_____
_____
_____
_____

3. Has a doctor ever told you that you have any of the following conditions?

Cancer	Y	N
Hepatitis C	Y	N
HIV/AIDS	Y	N
Heart Disease	Y	N
Liver Disease	Y	N
High Blood Pressure	Y	N
Diabetes	Y	N
Other chronic condition	Y	N

→
OVER

4. Do you have any food allergies? Y N

5. Are you **allergic** to nuts, food coloring, or yeast? Y N

If so, which one(s)? _____

6. Did you eat nuts prior to initiating dialysis? Y N

7. Do you have dentures? Y N

8. Will you have any problem eating either a few Brazil nuts, 3 gummy bears, or swallowing one tablet per day? Y N

9. Will you mind having three extra tubes of blood drawn on your normal blood draw days at the dialysis facility? Y N

10. Do you eat Brazil nuts on a regular basis? Y N

11. Please describe any other medical conditions that may affect your participation below (i.e. pregnancy, infections, allergies, etc):

_____
_____
_____
_____
_____
_____

Experimental Food (Brazil nut or dietary supplement): Place an "X" on the calendar for each day you ate the experimental food. If you forget to eat the experimental food, do not mark "X" the calendar.

October 2012						
SUN	MON	TUE	WED	THUR	FRI	SAT
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

November 2012						
SUN	MON	TUE	WED	THUR	FRI	SAT
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	

Experimental Food (Brazil nut or dietary supplement): Place an "X" on the calendar for each day you ate the experimental food. If you forget to eat the experimental food, do not mark "X" the calendar.

December 2012						
SUN	MON	TUE	WED	THUR	FRI	SAT
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31					

January 2013						
SUN	MON	TUE	WED	THUR	FRI	SAT
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

APPENDIX C

THREE MONTH DATA TABLES

TABLE 6. Antioxidant status outcomes by group over time[1]

Variable	Pill (n=7)	Nut (n=4)	Placebo (n=6)	p value[2]	Reference Range[3]
TAC (mM)					1.0-2.30
month 0	1.7 ± 0.3	1.7 ± 1.2	1.4 ± 0.6		
month 1	1.4 ± 0.6	2.2 ± 0.6	1.6 ± 0.7		
month 2	2.2 ± 0.8	2.1 ± 0.6	2.2 ± 0.8		
month 3	1.7 ± 0.8	1.8 ± 0.8	1.5 ± 0.6	0.267	
VitC (µg ascorbic acid/ml)					0.50-2.0†
month 0	0.30 ± 0.25	0.45 ± 0.44	0.17 ± 0.07		
month 1	0.27 ± 0.21	0.35 ± 0.30	0.17 ± 0.07		
month 2	0.26 ± 0.18	0.43 ± 0.42	0.20 ± 0.07		
month 3	0.28 ± 0.21	0.41 ± 0.34	0.20 ± 0.09	0.526‡	
RBC GSH-Px (U/g Hb)					20.0-71.0
month 0	75.5 ± 7.5	84.8 ± 14.7	62.0 ± 12.3		
month 1	67.4 ± 20.8	89.0 ± 12.3	60.8 ± 14.9		
month 2	73.3 ± 17.3	81.7 ± 11.1	61.4 ± 13.8		
month 3	76.2 ± 14.3	72.9 ± 11.4	61.8 ± 19.3	0.031	
Plasma GSH-Px (nmol/min/ml)					38.0-51.0
month 0	39.5 ± 6.0	46.1 ± 9.3	44.4 ± 13.7		
month 1	43.2 ± 10.7	44.6 ± 10.8	36.2 ± 10.1		
month 2	48.5 ± 11.6	42.4 ± 8.2	40.6 ± 6.8		
month 3	42.8 ± 11.8	37.6 ± 5.1	41.7 ± 6.6	0.065	

[1]Data presented as mean ± SD, n = 17. Univariate analysis indicated no differences at baseline. TAC, total antioxidant capacity; VitC, vitamin C; RBC GSH-Px, red blood cell glutathione peroxidase; Plasma GSH-Px, plasma glutathione peroxidase. Assessing for confounders (gender, age, EDW, and time on dialysis) revealed no associations.

[2] p value represents two-way repeated measures ANOVA for the group x time interaction. *Covariate controlled for in analyses. ‡Data not normally distributed hence p value for Kruskal-Wallis analyses change between 0 and 3 months

[3]Reference standard not established. Ranges indicative of healthy population in recent literature (†reference standard established).

TABLE 7. Cardiovascular disease outcomes by group over time[1]

Variable	Pill (n=7)	Nut (n=4)	Placebo (n=6)	p value[2]	Reference Range[†]
BNP (pg/ml)					<100
month 0	210.5 ± 87.4	266.4 ± 47.3	279.6 ± 134.2		
month 1	228.7 ± 84.3	229.7 ± 14.7	249.3 ± 88.3		
month 2	215.9 ± 114.1	242.2 ± 39.7	341.1 ± 124.1		
month 3	211.3 ± 79.6	241.5 ± 38.1	223.2 ± 31.1	0.387[‡]	
CHOL (mg/dl)					<200
month 0	133.8 ± 40.8	147.5 ± 3.5	149.1 ± 31.1		
month 1	152.3 ± 49.2	153.3 ± 28.7	145.8 ± 42.3		
month 2	149.8 ± 33.4	159.6 ± 23.7	154.8 ± 37.4		
month 3	144.2 ± 32.1	151.8 ± 13.2	151.1 ± 36.4	0.435	
HDL (mg/dl)					>60
month 0	46.5 ± 8.0	59.8 ± 38.1	40.7 ± 12.5		
month 1	48.1 ± 11.4	57.3 ± 26.7	40.1 ± 7.5		
month 2	44.1 ± 8.3	67.0 ± 43.9	42.5 ± 9.0		
month 3	44.1 ± 9.1	62.1 ± 39.2	39.9 ± 9.3	0.744[‡]	
LDL (mg/dl)					<100
month 0	75.7 ± 40.7	65.6 ± 13.3	88.4 ± 29.3		
month 1	87.8 ± 42.2	67.7 ± 18.5	84.3 ± 35.8		
month 2	86.9 ± 28.8	64.5 ± 25.8	88.9 ± 35.6		
month 3	80.3 ± 26.8	59.4 ± 23.4	87.3 ± 34.1	0.532[‡]	
TG (mg/dl)					<150
month 0	102.5 ± 29.7	142.8 ± 112.0	131.9 ± 44.8		
month 1	113.3 ± 41.6	184.6 ± 85.2	133.6 ± 67.7		
month 2	125.0 ± 34.8	178.7 ± 174.6	139.8 ± 47.2		
month 3	141.5 ± 48.5	183.8 ± 152.2	149.7 ± 46.3	0.449	
Systolic BP					<120
month 0	150.0 ± 22.3	129.8 ± 26.1	154.2 ± 24.8		
month 1	141.9 ± 25.0	121.0 ± 15.6	147.5 ± 24.2		
month 2	162.7 ± 30.7	133.5 ± 7.3	141.3 ± 28.2		
month 3	149.6 ± 25.8	143.3 ± 13.5	141.7 ± 19.5	0.271	
Diastolic BP					<80
month 0	71.7 ± 9.2	82.0 ± 30.1	79.5 ± 19.0		
month 1	74.9 ± 25.1	76.3 ± 14.4	84.0 ± 23.5		
month 2	76.1 ± 9.4	81.8 ± 17.8	81.2 ± 12.1		
month 3	78.7 ± 16.9	84.5 ± 17.4	77.0 ± 14.2	0.581[*]	
TCFA (ohms)[3]					19.0-30.0
month 0	31.8 ± 4.0	31.3 ± 1.6	31.7 ± 6.4		
month 1	33.0 ± 5.1	28.8 ± 5.7	32.4 ± 5.4		
month 2	31.1 ± 5.8	30.9 ± 2.0	31.2 ± 4.7		
month 3	31.4 ± 3.7	29.5 ± 4.0	26.9 ± 5.0	0.053	

[1]Data presented as mean ± SD, n = 17. Univariate analysis indicated no differences at baseline BNP, brain natriuretic peptide; CHOL, cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; BP, blood pressure; TCFA, thoracic cavity fluid accumulation. Assessing for confounders (gender, age, EDW, and time on dialysis) revealed 2 association: HDL and gender (M: 37.3 ± 12.1, F: 56.8 ± 22.7, p=0.013) and Diastolic BP and age (r=-0.561, p=0.004). [2] p value represents two-way repeated measures ANOVA for the group x time interaction. *Covariate controlled for in analyses. [‡]Data not normally distributed hence p value for Kruskal-Wallis analyses change between 0 and 3 months. [†]reference standard established. [3]n=14